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FOREWORD

Agriculture production increased significantly in the second half of the past century as a result of intensification through increasing external inputs first of all energy and agrochemicals per unit of land. To meet the challenges of increasing food demand, ways should be found to improve the productivity, profitability and sustainability of the agricultural and forestry systems.

Industrial agricultural system has led to irretrievably changes in the landscape diversity, soil quality, environment integrity, and natural resource base. This has resulted in major questions world-wide in relation to the sustainability of agricultural production systems. At the field level, the optimization of resource use efficiency is a key to issue to achive the different goals with respect to food supply, income and protection of the environment.

The most significant damage to natural ecosystems and the environment as a whole is caused by habitat conversion and corresponding climate change, loss of biodiversity and ecosystem functions, soil erosion and degradation, and pollution from fertilizers and pesticides.

Concepts in plant protection have changed in past decades from exclusion or destruction of pest to pest management. Serious problems with pesticides, rapid development of pest resistance, environmental effects of pesticides, and high costs led to the development of new approaches and techniques in pest management based on improved knowledge of pest dynamics and their natural enemies, and the interaction between the pest and the crop.

Many of the papers included in the present Proceedings identify a number of approaches and market-based inventives that would encourage producers to achieve entirely new levels of performance, and as a result raise the awareness of governments, and other stakeholders in the agro-food system. Some governments, too are experimenting very innovative approaches to support or encourage sustainable agriculture. One of the goals of the sustainable agriculture movement is to create farming systems that mitigate or eliminate environmental harms associated with industrial agriculture. That aim can be realized only with adapted agricultural practices to the different agro-ecological conditions (cf. different regional characteristics, soil types, adapted cultivars for low-input or organic production). For our conditions it is important for the conservation of the ecological environment to develop organic production.

The result reported here will be also significant in the dissemination of knowledge to the wider audience about the importance of agriculture and food science, one of the most important strategic scientific areas of the European and many national research strategies.

The 6th International Scientific Agriculture Symposium “Agrosym 2015” made an important contribution to the agriculture practice in different areas. During the four-day symposium (15-18 October 2015), approximately 750 papers were presented to more than 800 participants representing more than 70 countries.

The symposium was made possible through the commitment and contributions of a wide range of partners and cosponsors. We were encouraged by symposium success.

This publication comprises an edited selection of the many papers submitted to the symposium.

It remains only for me to thank all those who have helped to make this Symposium such a great success.

Much appreciation is due to the authors and reviewers of all papers submitted and presented at the symposium, as well as to all symposium participants whose ideas and contributions ensured rich and lively discussion during the various sessions.

East Sarajevo, 03. October 2014.
Dušan Kovačević, PhD
Editor in Chief

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KEYNOTE PAPERS

Review paper
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TOWARDS A NEW PARADIGM OF INNOVATION IN THE BIOECONOMY

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Introduction and outline

We live in troubled times. Inequalities in income and wealth are growing nearly everywhere nationally and transnationally (Picketty, 2014; Varoufakis *et al*, 2014), as are migration and refugee streams resulting from failures of political, social and economic systems and the collapse of the climate regulation system. The neoliberal hegemony has so far triumphed, and private capital is penetrating formerly public spheres, and generating further inequalities. However, the system is 'broke', and it is up to the intellectual community to join with civil and political society in the search for alternatives. If both the extremes of soviet communism and neo-liberal finance capitalism are dead, we must search for a range of 'third way' solutions in which the Nordic model has something to offer (Bryden *et al*, 2015). Without important changes, the world will become less, and not more, sustainable. We, as social and indeed natural, scientists can no longer afford to adopt the fiction of objectivity and avoid normative questions, as Weber famously advised us to do; we need to address the norms and values that underpin our work (Bryden and Gezelius, 2014).

An increasing number of scholars believe that innovation, innovations systems, and innovation policies and practices are adding to these problems rather than solving them (STEPS, 2010). This is a huge challenge for the innovation community in general, and to innovation scholars in particular.

In this paper I suggest that a new paradigm of innovation is needed if these concerns are to be addressed, especially perhaps in relation to the bioeconomy, but also in other fields. I further suggest that the new paradigm is reflected in some key examples of locally generated and initiated innovation systems in rural regions. The order of the discussion, and questions raised, are as follows:-

- What is the bioeconomy and what are its special characteristics
- What is innovation?
- Who is innovation for?
  - The '3 Ds' – Direction, Distribution and Diversity
  - The TBL – Economic, Social and Environmental
  - The HRB-TBL – Human Rights governed TBL
- Why we need a new paradigm?
- Can we see signs of the new paradigm in the 'real world'?
- How can we stimulate and shape the new paradigm through praxis and policy?
- Concluding remarks

The Bioeconomy

Our focus here is on the bioeconomy because the wider bioeconomy has a number of important particularities, notably

- It is a source of basic needs for all humanity – especially food, water, shelter;
- It is associated with many public and quasi public goods, notably climate regulation, landscapes, biodiversity;

- It is closely linked to a number of important human rights, notably the rights to an adequate diet, clean water, and perhaps in future energy;
- Bioeconomy is part of the transition to a low carbon economy, involving the replacement of fossil-based fuels, plastics, building materials, pharmaceuticals etc.
- One of the key natural resources on which it is based – land – is limited in supply (cannot be easily added to) and variable in quality, as well as being closely linked to important social systems of ownership and use rights.

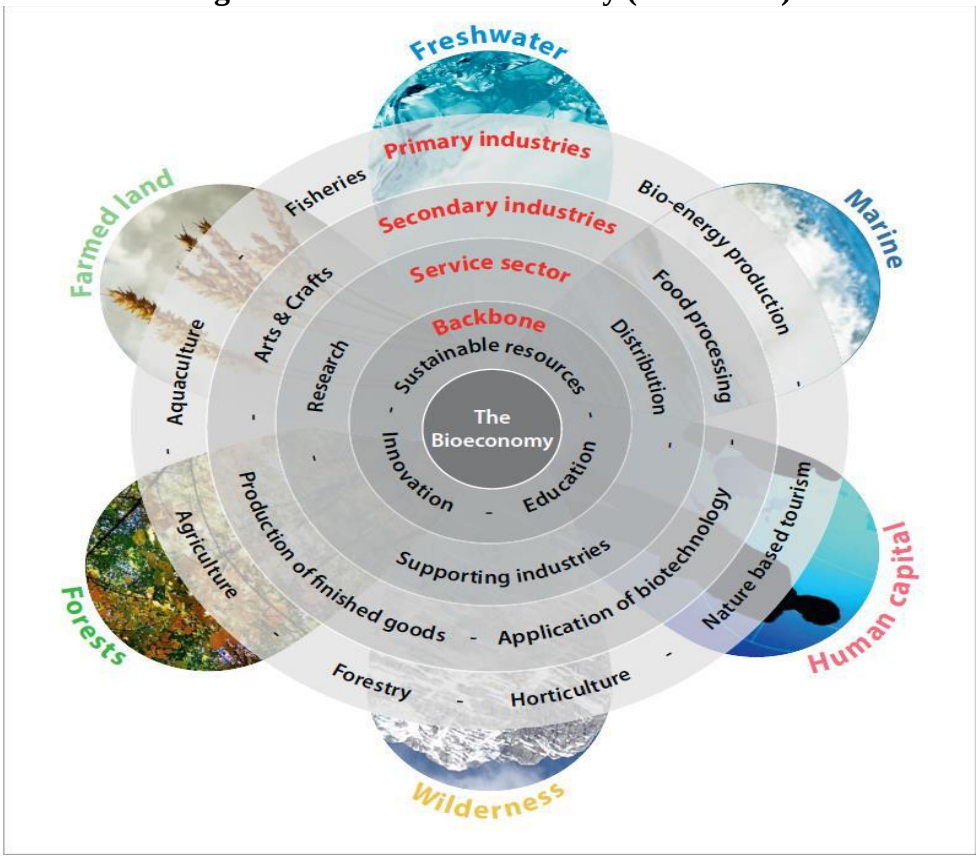
In NIBIOs grounded and inclusive innovation research group[1], we define the bio-economy widely, to include, in principle, all the living natural resources of land, air and sea that are transformed into useful commodities or public goods and services (including 'ecosystem services'), together with their related upstream and downstream industries. Agriculture and its related upstream and downstream sectors, the main focus of this conference, all lies within the bioeconomy. As social scientists, out key interest lies in human behaviour and decisions relating to this bioeconomy, and especially concerning changes defined as 'innovation', and related institutions, policies and governance, and in conflicts of use. These conflicts are important, since the biomass has alternative uses with different social and market values. One way to deal with such conflicts – for example between energy and food – is to adopt what is called the 'cascading' principle, which means taking the highest value uses first and using waste or residues for the lowest value uses. Here social scientists must help decision-makers and citizens penetrate the term 'value', and the determinants of value when market price is taken as a surrogate for 'value'.
The broad definition of the bioeconomy we adopt is reflected in the mostly recent bioeconomy strategies of a range of countries, the EU, and also recent documents of the OECD. The following diagrams illustrate the cases of Finland (mainly forest-based bioeconomy) and Iceland (mainly marine-based bioeconomy).

Figure 1: The Finnish Bioeconomy Strategy

"Bioeconomy refers to an economy that relies on renewable natural resources to produce food, energy, products and services. The bioeconomy will reduce our dependence on fossil natural resources, prevent biodiversity loss and create new economic growth and jobs in line with the principles of sustainable development."

[1] At one time called NILFs 'Green Innovation' Group. See http://www.nilf.no/green_innovation_research/
NILF joined Bioforsk and Skog og Landskapp Institutes to form the Norwegian Institute for Bioeconomy Research (NIBIO) in July 2015. The websites are not yet harmonized.

Figure 2: Icelandic Bioeconomy (Matis Ltd.)



Our main focus in this paper is the land-based bioeconomy, but in our view the new paradigm for innovation is probably one that is universally desirable in all fields.

Innovation

We must think of *innovation* broadly – not only *technological* innovation, and also not as equivalent to *invention*. Of course invention and technology are important, but if indeed the system is ‘broke’, then we desperately need institutional and policy innovations. It is not innovation or invention *per se* that produce unsustainable outcomes, but human behaviour and the institutional and governance systems that guide it (Bryden, 1994). Moreover, the problems of complexity, implicit in the goals of sustainable development, which cut across disciplinary boundaries, also demand multiple solutions and adaptations to different needs and contexts[2]. This is especially so in the bioeconomy based on natural resources, where multiple natural conditions determine the production possibilities, productivities, and interactions with society and environment. One size does not – and cannot – fit all needs and circumstances.

Innovation is different from *invention*. In particular, Innovation typically involves *adaptation* of existing techniques or institutions to local (economic, social, cultural, geographical, physical, ecological) conditions, needs, etc. While we generally, but not always correctly, think of *invention* as the solitary work of a scientist , craftsman or entrepreneur, innovation is commonly a collective process involving a range of actors in ‘doing using and interacting’ (‘DUI’). Interaction typically takes place between public institutions, universities or research instituted, raw material suppliers, craftsmen and enterprises transforming raw materials, and final users of the goods and services produced. Research has demonstrated that innovations typically involve two key modes working together, namely science, technology and

[2] Delvalle(2015) cites Ashby’s (1956) Law of Requisite Variety in his discussion of the governance problems around complex issues.

innovation ('STI') and DUI (Jensen et al, 2007). Here the growing focus of research has been on 'innovation systems', first at national level (Lundvall, Freeman, Nelson, Fagerborg among others), but later at regional and local levels (Edquist, Cooke et al, and others). The innovation systems research focused on the nature and process of *collective learning* and *forms of knowledge*. Within that, the regional and local innovation systems research focuses on the *embedding* of innovation systems within specific regional and local contexts. Our main focus in this paper is on *local* innovation systems, and especially 'grass roots', 'inclusive' and 'participatory' innovation in its various forms.

We further argue that such local innovation systems are 'social' in the sense that they are collective learning systems. Later we add the further notion that they are also 'social' in at least some of their key objectives. They are also dynamic, in the sense of all learning systems – the various conditions under which they are functioning alter through time, and they in turn have new issues and questions to solve; feedbacks from initial changes also cause internal adjustments and innovations. Innovation thus needs to be seen as an on-going process, rather than a one-off occurrence.

Innovation and sustainability

The STEPs[3] manifesto (STEP 2010), the work of the collaboration of scholars from many countries, argued that radical changes in the policy and institutional framework were needed if the problems of sustainable development were to be tackled. Specifically we needed to address the problems of the Direction and Diversity of Innovation, and the Distribution of the benefits arising from it – the so-called Three D's. This is closely related to the notion of the '3 Ps' – People Planet and Profit – and to that of the 'Triple Bottom Line' or TBL (Elkington, 1997). However, as Bryden & Gezelius (2014) argue, the 3D's contain an ethical imperative, whilst the TBL is based on consequentialist ethics. Ethical issues are very important in the bioeconomy, for several reasons, but especially because many basic needs are goods and services from the bioeconomy, or closely linked to it, notably food, water, housing and energy. Moreover, food and water are codified in Human Rights legislation. This issue is further discussed below.

The growing concern about the link between innovation and processes of social inequality and all levels is indicated by the international network of scholars within the Globelics group, who study innovation systems. Globelics recently held an international conference on Innovation and Inclusion in Cuba (September 2015). The high level of submissions from all parts of the world indicates the strong degree of interest in the issue of whether innovation policies and institutions, as well as innovation systems, can help to address the problems of social inequalities, whatever their source.

There has also been a growing interest in innovation and innovation systems within the EU. The SCAR committee report (2012) emphasised the role of innovation platforms[4], as did the discussion of European Innovation Partnerships in Innovation Europe. This was followed by the current R&D programme H2020, which has a large focus on innovation including social innovation, and which was certainly influenced by the SCAR report. In one sense innovation platforms can be seen as social innovations, because they explicitly recognise the importance of social processes of collaboration and learning in creating (product and process) innovation.

[3] STEPS Centre at Sussex University, a collaboration between SPRU (Science Policy Research Unit) and IDS (Institute of Development Studies) supported by the UK Economic and Social Research Council.

[4] Innovation platforms are not a new idea – Roling and Jiggins (1994) worked on them, drawing on the soft systems work of Checkland (1981) and Checkland & Scholes (1991), discussed in Bryden 1994. However, what is new is the debate on the impacts and objectives of innovation stimulated by the STEPS *New Manifesto* in 2010 and subsequent work in this area.

However, since social innovation is most usually treated as a sub-category of innovation, and one that is mainly concerned with the delivery of social services[5], we prefer to address the general innovation discourse in this paper.
Given this background, there are three further questions we want to address in this paper. Firstly, why do we need a new paradigm? Secondly, what would the new paradigm look like in practice, given our revised norms? Thirdly, can we observe instances of innovation systems that illustrate the new paradigm in practice?

Why we need a new paradigm?
A set of more or less tacit normative value premises underpins most of today's innovation discourse, and is taken for granted by policy makers (Bryden & Gezelius, 2013, 2014, 2015; Bryden et al, 2015). It is also, for the most part, silently and uncritically adopted by scholars. In particular, a widely adopted premise is that a key purpose of innovation is to *promote economic growth and to increase the international competitiveness of national industries*. Founding on this premise, it is the ability to promote growth and competitiveness that makes innovation a 'good thing' and that justifies public funding of innovation related research and development. The evidence base is thus influenced, even determined, by these tacit norms and values: this consists, mainly, of numbers on public and private expenditure on research & development, and the numbers of patents filed, as measures of input and output respectively. This is what represents the 'old paradigm' because it focuses on only one of several sources of knowledge needed for the processes of co-learning that are implicit in most innovation systems.
But suppose we challenge and replace these norms with others? In particular, norms based on premises about sustainable development and human rights?
STEPs' *New Manifesto* emphasises that development and innovation should pursue poverty reduction, social justice, and environmental sustainability.
To achieve this, innovation should be driven by those in the greatest need of it. Consequently, innovation should be driven from the bottom-up, rather than by politicians and scientists who promote ready-made solutions that disregard local needs and contexts. Innovation processes should thus include, especially, poor and underprivileged groups. Their guiding '3 Ds' are about the *Direction* of Innovation and its link to the *Distribution* of income and wealth, and to *Diversity* of solutions to practical problems.

- **Direction** – what direction will this or that type of innovation take us? Some pathways – like highly specialised, capital-intensive, centralised, large-scale and long lead-time nuclear infrastructures – can 'crowd out' alternatives. Where should innovation resources be focused?
- **Distribution** – what will be the impacts on distribution of income and wealth? Will the innovation help the poor? How? Many scholars of development do not accept the hypothesis that this question does 'not matter' because wealth will 'trickle down' no matter where it is created, since the hypothesis is contradicted by the evidence and has no robust theoretical underpinning in any case.
- **Diversity** – diversity is a strength (not to put 'all eggs in the same basket'; letting 'a thousand flowers bloom').

Triple bottom line (TBL) accounting is a principle for business ethics developed largely within a rich country context (Elkington, 1997). Its basic idea is that business results should

[5] Much of the discourse relates to the so-called 'social economy', in which much of the activity concerns delivery of social services that were formerly delivered by public – often local – authorities within the framework of the welfare state. This focus is however too narrow for our purposes.

be measured not only in terms of profit, but also in terms of social consequences and environmental effects. It is very similar – even analagous - to the idea of PPP, or 'people, planet, and profit', widely adopted by environmental interests, for example.
Despite obvious similarities, the New Manifesto and the Triple Bottom Line build on different ethical foundations. The STEPS manifesto brings in a strong element of duty ethics, focusing on social justice and addressing human rights such as the poverty issue. Triple bottom line thinking is fundamentally consequentialistic because of its focus on the measurement of outcomes. Social justice is by nature an *obligatory* normative standard. Human rights are even more so; *they are not something that can be traded off against other benefits.*

To conclude this section, we need a new paradigm of innovation to

- change the *objectives of innovation*
  - three Ds + TBL = HRB-TBL
  - "innovation as if people mattered"
  - "inclusive innovation"
- change *innovation praxis*
  - from STI to STI plus DUI
  - from top-down to bottom-up
  - from exclusion to inclusion
- change *innovation policies*
  - from science-led to actor-led
  - from property rights to common property
  - from private interest to public interest

What should the New Paradigm look like?
At this stage we can briefly contrast the 'old' and the 'new' paradigms. The 'old paradigm' of innovation is primarily characterised as innovation serving the private sector in its quest for international competitiveness through improvements in the productivity of labour and capital. State support for innovation has become almost wholly captured by this normative and largely tacit objective, which is nowadays barely discussed. In contrast with Polanyi's notion of the market being 'within' the State, the State is now within the market, a very powerful belief in western market economies, especially the 'anglo-saxon' and lately neo-liberal countries of the UK, the US, Canada and Australia. The State is seen as 'subservient' to capital, serving its needs, rather than protecting citizens from the impacts of capitalism, for example on inequalities and the environment. Capital is in fact directing the focus of R&D through its close relationship with national and transnational policies, and firms and individual entrepreneurs and 'innovators' are seen as the main actors and policy targets. The main and often the only indicators used to measure the 'strength' of innovation, or the rank order of states, are the number of patents registered and the expenditure on research and development. The outcomes may be evaluated by growth in productivity or GDP.
The 'new paradigm' stands in stark contrast to this. Based on the previous, normative, analysis, we argue that innovation should serve citizens, and the State's activities and policies in relation to innovation should be based on the needs and priorities of citizens as expressed through civil and political society. The market, and market actors, are of course part of that civil and political society, but we follow Polanyi (1944) in arguing that the market is within the State, rather than *vice-versa*. Innovation is thus driven by societal problems and priorities – especially 'sustainable development'. To summarise, the New Innovation Paradigm involves:

1. the state serving citizens; the market is within and not above the state;

2. the objectives are first and foremost *social; they are concerned with human wellbeing and the natural environment that sustains it; where these are codified in human rights they give priority to these;*
3. research and innovation as a *multi-actor collective learning process* which is *inclusive* in the sense that it includes all those with an interest in the outcomes, including those normally excluded from such processes. This is wider than the notion of 'including users' where 'users' are considered as 'customers';
4. innovation policies target and encourage multi-actor innovation platforms that are inclusive, we call these 'Grounded Innovation Platforms' or 'GRIPs' (Bryden et al, 2013; Bryden and Gezelius, 2014; Bryden et al 2015)
5. the outcomes of innovation are assessed by TBL and Human Rights impacts.

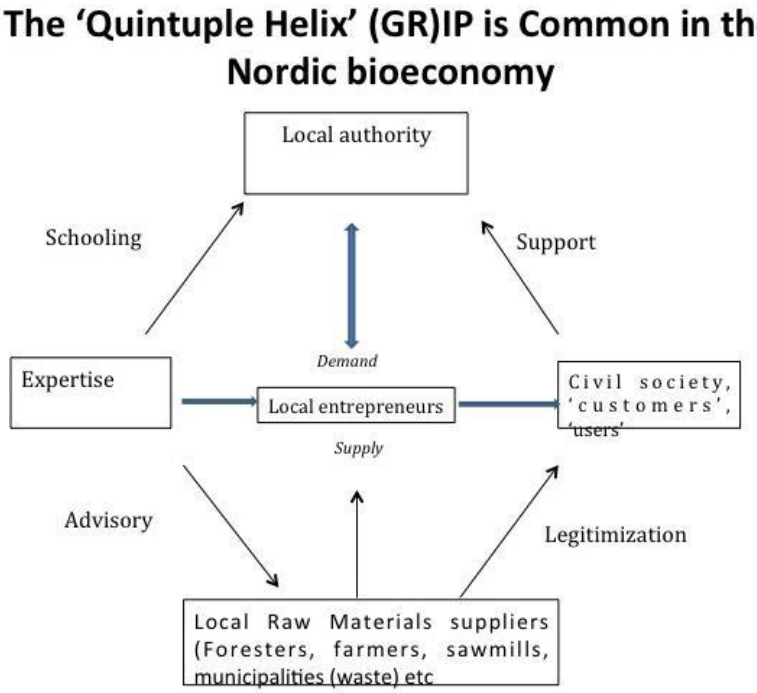
Can we see signs of the 'new paradigm' in the 'real world'?

Sheik (2015) discusses 'grass roots' innovation in Indian 'informal' sector. He cites Gupta's (1999) attempt to conceptualise grassroots innovations in India, in which he "maintains that technological solutions at the grassroots level seem to be more environmentally sustainable, local in nature, developed through bottom up approach and suited to the local communities". In his paper, Sheik focuses on two Indian institutions focused on the poor, and on action research at the grass roots level involving the 'blending' of formal and informal knowledge systems. These are the National Innovation Foundation (NIF) and Barefoot College (BC). NIF, in collaboration with Shodyatras and its volunteers at Honey Bee Network "claims to have build up a database of more than 200,000 ideas, innovations and traditional knowledge practices from over 555 districts of India

To us the GRIP is one concrete expression of the New Paradigm in action in the Nordic context, but there are many terms for the same kind of structure. A GRIP is firstly an innovation platform in the sense that it is a grouping of actors who come together because they have a shared goal of changing something together. The object can be technology, but it can also be a policy or a practice going beyond technology. It is a means of collective learning by those whose lives are most directly affected by the outcomes, and so it is a 'participatory' or 'bottom up' process. It also builds on the existing experience, knowledge and evaluations of the actors. It may or may not be inclusive, and it may or may not have HR-TBL outcomes – neither can be assumed, and both are empirical questions[6]. But we follow Gupta in hypothesising that such new paradigm institutional forms are likely to be more inclusive and more likely to have HR-TBL outcomes than those of the old paradigm.

Our own research in the TRIBORN project can be used to illustrate GRIP like structures. In it we have identified a form of 'quintuple helix' structure active in the bioeconomy at local levels in Norway, Sweden and Finland. That is to say there are five distinct types of actor involved, notably the suppliers of raw materials (typically foresters or farmers, but also sawmills, food processors and other sources of 'waste' biomass materials); the firms that transform those materials into energy (district heating, electricity, biofuels, biogas, pharmaceuticals, bioplastics and chemicals etc); the local authorities that create local regulations and so engage in 'market-making' as well as being 'responsible' to their electorate and therefore concerned with legitimacy, fairness and other ethical and moral questions as well as local development and employment issues; one or more sources of formal knowledge, in some cases universities and research institutes, in others knowledge intermediaries such as extension agents, consultants, etc. ; and finally, the citizens either as 'customers' or through their civil society organisations. The following diagram illustrates such structures:-

[6] Research has shown that such 'bottom up' structures can be 'captured' by elites, for example.



There are also many examples of similar structures from around the world. Some are documented in the background work for the New Manifesto. Others are discussed by Fressoli *et al* (2014), and Sheik (2015) mentioned earlier. Alfredo del Valle from Valparaiso in Chile recently discussed a paper called : "Building a rural future in Valporaiso, Chile via Participatory Innovation. Methodical governance of complexity as a seed for post-neoliberal policy" in which he discusses the structure of this new governance system. Since the local governance system he describes incorporates government, the productive sector, business-associations, the knowledge sector and NGOs, we can think of it as a form of GRIP. The local action groups of the EU LEADER programme had similarities to a GRIP in its earlier manifestations prior to 'mainstreaming' (Bryden, 2011). In more general terms, the move to more 'inclusive' innovation in terms both of its objectives and its institutions is an on-going topic of discussion and research within the GLOBELICS group, thanks to the work of young scholars like Judith Sutz, Gaby Bortz, Mariano Fressoli, and Susan Cozzens, to name but a few.

To some extent at least, the European Innovation Partnerships (2010), elaborated for agriculture in the EU report of the Standing Committee on Agricultural Research (SCAR) 2012, opened a door to the new paradigm, even if the arguments were somewhat different (if also relevant). The EIPs and the SCAR report undoubtedly influenced the thinking behind Horizon 2020. However, the focus of the EU has mainly been in EU competitiveness and technology development, and our arguments and proposals go far beyond these issues.

Conclusions

1. *The world needs a New Paradigm of Innovation (NPI)*
    a. To address growing global problems of inequalities and exclusion, climate change and its impacts
    b. To shift the normative goals of innovation and innovation policy
2. *There are many examples of institutions from different parts of the world that appear to illustrate the New Paradigm, and policies moving towards their support*. At the moment, though, they are a form of experiment

   a. We need to study them carefully, and learn from processes and outcomes
      i. How do GRIPs form, how do they learn and adapt, are they temporary or long-lasting?
      ii. What kind of formal or informal governance and/ or organizational structures do they have, and how do these influence things like learning processes, longevity, and outcomes?
      iii. What are their objectives and are these concerned with HR-TBL outcomes?
      iv. Do innovations through GRIPs produce HR-TBL outcomes?
3. *Such experiments give us grounds for hope in the quest for more sustainable human development.*

We will be continuing to work on these issues in future both in relation to the bioeconomy, and beyond it, and hope that others will join in this endeavour in their own fields. We will also work with policy makers at all levels to find new ideas about how GRIP-like structures can be fostered without becoming controlled and inhibited by the bureaucracy and technocracy that kills so many creative ideas and structures. What is sure is that public policies towards the stimulation of innovation need to change if innovation is to contribute fully and adequately to social, environmental and economic goals of sustainability and to human rights.

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150 YEARS OF THE GENIUS OF GENETICS

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Abstract

In 2015 world is commemorating 150 years since Gregor Mendel presented his lectures Experiments in Plant Hybridization (*Versuche über Plflanzen-hybriden*), on the 8th of February and March 1865. He presented, for the first time, the results of his research into breeding peas and other plants that he had been performing for a number of years in the gardens of the Augustinian monastery in Old Brno at a meeting of the Natural Science Society in Brno (*Verhandlungen des naturforschenden Vereines in Brünn*).

Using the garden as a genetics laboratory, Gregor Mendel could manipulate and select for certain traits to formulate and test out his hypotheses about mechanisms of inheritance and distribution of traits in offspring. In order to trace the transmission of traits, he chose seven pea traits that were expressed in a distinctive manner, such as plant height (short or tall), seed color (green or yellow), etc. The F_1 of crossing varieties that differed in one trait- for instance tall x short, displayed the trait of one variety but not that of the other. He observed that the plant height was a trait that was passed on to progeny independently from other traits. In Mendel's terms, one character was dominant and the other recessive. The recessive character reappeared in F_2, and the proportion of offspring bearing the dominant to offspring bearing the recessive was very close to a 3 to 1 ratio.

After seven years of research and analyses of thousands pea plants, Mendel′s observational talent and mathematics knowledge were base for the defining of three principles, often known as Mendel's principles of heredity- Law of dominance, Law of segregation and Law of independent assortment, which are the essential for the Mendelian genetics and heredity. Remarkably, Mendel described elements of DNA-based inheritance in the absence of any knowledge of the molecular biology behind it. His important contributions to the field of genetics continue to influence modern scientists and every geneticist or genetics student has heard his name.

Although initially well-received, Mendel's work was not given proper appreciation during his lifetime, even after it was published in 1866. In fact, over the next 35 years, this paper was only cited three times. The genetics became more important at the beginning of the 20th century when three different research groups (Dutch botanist and geneticist Hugo de Vries, German botanist and geneticist Carl Erich Correns, and Austrian botanist Erich von Tschermak with their co-workers) independently re-discovered Mendel's Laws. Biologist William Bateson became the strong supporter of Mendel's theory and later zoologist and geneticist Thomas Hunt Morgan located the genes on the chromosomes. As the architect of genetic experimental and statistical analysis, Mendel remains the acknowledged father of genetics.

Key words: *Gregor Mendel, genetics, inheritance, dominant, recessive.*

Introduction

Since the beginning of human history, people have wondered how traits are inherited from one generation to the next. The fact that living things inherit traits from their parents has been used since prehistoric times to improve crop plants and animals through selective breeding. Although children often look more like one parent than the other, most offspring seem to be a blend of the characteristics of both parents. Centuries of breeding of domestic plants and animals had shown that useful traits - yield of wheat, speed in horses, larger fruits in crops, protein content in milk - can be improved by controlled mating. However, there was no scientific way to predict the outcome of a cross between two particular parents.

A number of hypotheses were suggested to explain heredity. During the 19th century, prior to the discovery of genetics, many biologists support to the idea of blending inheritance (Jenkin, 1867). Blending inheritance was merely a widespread hypothetical model, rather than a formalized scientific theory since it was never formally presented to a scientific community nor published in any scientific journals. By this theory inherited traits were determined randomly, from a range bound by the homologous traits found in the parents. For example the height of a person, with one short parent and one tall parent, was thought to always be of some intermediate value between its two parents' heights. The shortcoming to this idea was in how it required the person of intermediate height, in turn, to then become one of the limiting bounds (either upper or lower) for future offspring, and so on down the entire lineage. Thus, in each family, the potential for variation would tend to narrow, quite dramatically, with each generation, and it would go for the entire population with every trait. If blending inheritance were true, in this example, all members of a species would eventually converge upon a single value for height for all members, variation disappeared, every generation should be more uniform than the previous one. By now, all individuals should be as indistinguishable as clones. Blending inheritance failed to explain how traits that seemingly disappeared for several generations often reasserted themselves down the line, unaltered. Blue eyes and blond hair, for example, often could disappear from a family's lineage for several generations, only to have two brown-haired, brown-eyed parents give birth to a blond, blue-eyed child. If blending inheritance were fact, this could not be possible.

In 1868 in England, Charles Darwin proposed his pangenesis theory (pan- whole, genesis-origin) to describe the units of inheritance between parents and offspring and the processes by which those units control development in off spring (Darwin, 1868). Indeed, pangenesis theory originated from the claim that characteristics acquired during an organism's life were heritable. A theory of inheritance of acquired characteristics (IAC) had persistent for almost two thousand years, since Greek antiquity. Hippocrates, who lived during the fifth to fourth centuries BC in Greece used this theory to explain why some people had longer than normal heads. Many scientists and philosophers in the eighteenth and nineteenth century favored some form of IAC theory, including Jean-Baptiste Lamarck in Paris, France. Lamarck stated that all the characters acquired during an individual's life transmitted to their offspring.

Darwin propose the concept of gemmules, which he said referred to hypothesized small particles of inheritance inside cells. Darwin argued that, in what he called higher animals or plants, every cell in their bodies emitted small particles, which were units of heredity, that he called gemmules. The gemmules could either circulate and disperse in the body system, or they could aggregate in the sexual cells located in reproductive organs. As hereditary units, the gemmules transmitted from parents to offspring, where they developed into cells that resembled the parents' cells. It was not sexual cells alone that generated a new organism, but rather all cells in the body as a whole. The theory suggested that an organism's environment could modify the gemmules in any parts of the body, and that these modified gemmules would congregate in the reproductive organs of parents to be passed on to their offspring. In sexual reproduction, gemmules from both parents blended in the sexual organs to be passed to

the offspring With the term pangenesis, Darwin suggested that all parts of the parents could contribute to the evolution and development of the offspring. Darwin's theory of pangenesis gradually lost popularity in the 1890s when biologists increasingly rejected the theory of inheritance of acquired characteristics (IAC), on which the pangenesis theory partially relied.

Around the turn of the twentieth century, biologists replaced the theory of pangenesis with germ plasm theory and then with chromosomal theories of inheritance, and they replaced the concept of gemmules with that of genes. Weismann stated that small units of materials in the cells of organisms pass from parents to their offspring (Winther, 2001). Nonetheless, Weismann argued that only the hereditary material in the sexual cells, or germ cells, could transmit to offspring. Weismann called his theory germ-plasm theory. Principles of the germ-plasm theory gradually replaced Darwin's pangenesis theory, but biologists adopted little of Weismann's terminology.

The particulate hypothesis states that parents pass on to their offspring distinct factors that retain their offspring while the blending hypothesis states that parents′ hereditary material blends in their offspring.

In the 1860s in Brno, Austrian Empire, which later became the Czech Republic, Mendel had studied how heritable factors in sexually reproducing plants behaved across generations, and he had inferred laws to describe those behaviors. Mendel did not study the actual units of inheritance, but only the phenotypes or traits hypothesized to develop in organisms that had those factors. With his laws, Mendel could predict phenotypes among the offspring from data about the phenotypes of parents. Mendel's laws, unstudied for decades, were rediscovered in 1900, and Walter Sutton in the US and Theodor Boveri in Europe soon paired those laws to the mechanistic descriptions of how chromosomes behaved in replicating cells, creating a chromosomal theory of inheritance (Satzinger, 2008). Mendel's hypothesis states that parents pass on to their offspring separate and distinct factors (today called genes) that are responsible for inherited traits. Mendel stressed that these heritable factors retain their identity during next generations.

Following Bateson in England and Johannsen in Denmark, most biologists eventually rejected theories of the inheritance of acquired characteristics and Darwin's pangenesis theory, and they used the term gene to refer to those things that Mendel had called factors (Bateson, 1900; Johannsen, 1909). Scientists in the early and middle decades of the twentieth century worked to fit the chromosomal and genetic theories of inheritance with Darwin's theory of the evolution of species by natural selection.

Education and early career of Johann Mendel

Johann Mendel was born 1822 in a farmer′s family in Hynčice about 120 km north of Brno. He was the son of Anton and Rosine Mendel, and had two sisters; older Veronika and younger Theresia. From his mother Johann Gregor inherited some of his character traits, for he seems to have been good-natured, quiet, and modest. Probably his talent came also from the mother's side of the family. Anton Mendel had a great fondness for fruit-growing. They lived and worked on a farm which had been owned by the Mendel family for at least 130 years. During his childhood Johann helped his father farm their land working as a gardener and studding beekeeping, but he was not destined to be farmer. On the farm Johann developed his love for the science.

Johan began attending the village school as soon as he was old enough and his teachers noticed that he was an exceptionally intelligent boy. Johann′s teachers recommended him to an upper elementary school in Leipnik. This was a school where exceptional students were sent to prepare them for the gymnasium. The gymnasium (grammar school) was a school that provided an education for the best students, which were selected to go on to study

an university. Later, when Johann was 11 years old, he attended Gymnasium in Opava in 1834. Johann's parents had to deal with the fact that Johann would not be taking over the family farm when his father grew older. In 1938 Johann's father was seriously injured while working and that situation places a lot of stress on Johann, because his parents were no longer able to pay for any of his schooling, leaving him to support himself entirely. Due to that Johann become quite sick and stayed in bed for four months. To provide money for studying Johann decided to study to become a teacher for tutoring his classmates. He graduated from the gymnasium in 1840.

Johann wanted to continue his education at a university and prerequisite for that was two year philosophical study. From 1840 to 1843, he studied religion, philosophy, ethic, pedagogy mathematics and physics at the University of Olomouc Faculty of Philosophy, taking another year off because of illness. When Mendel entered the Faculty of Philosophy, the Department of Natural History and Agriculture was headed by Johann Karl Nestler who conducted extensive research of hereditary traits of plants and animals, especially sheep. Johann had the problems with his health whenever he was faced with difficult circumstances. He also struggled financially to pay for his studies, and Theresia gave him her dowry. Later he helped support her three sons, two of whom became doctors. He was excelled in physics and mathematics. Johann Mendel no longer wanted to have to struggle.

Indeed, Friedrich Franz, his professor of physics who was a priest, urged Johann to become a monk. For Mendel that was the only way for obtaining education since monasteries were known to be centers of learning. Johann Mendel in late 1843, at the age of twenty one, joined the Augustinian monastery of St. Thomas and began his training as a priest; born Johann Mendel took the name Gregor. Gregor Mendel was ordained into the priesthood in August 1947. As a priest Mendel found his parish duty to visit the sick in hospital. It quickly become clear that he was not fit for these duties because whenever he visited the patients he became very upset. Mendel wanted to teach. Head of the monastery Abbot Napp found him a substitute-teaching position at Znojno, where he proved very successful in teaching mathematics and literature at the high school. Mendel began teaching in 1849, even he did not yet have a formal document from a university approving him to do so. In 1850 he failed the oral part, the last of three parts, of his exams to become a certified high school teacher. From point of Mendel it was debacle, but from point of genetics it was good that he did not pass the exam. If he passed Mendel probably would be happy teaching at the Znojno high school for the rest of his life and probably would never work with pea.

In 1851 Mendel was sent to the University of Vienna to study under the sponsorship of Abbot Napp so that he could get more formal education. As at Olomouc, Mendel devoted his time at Vienna to physics and mathematics, working under Austrian physicist Christian Doppler and mathematical physicist Andreas von Ettinghausen. He also studied the anatomy and physiology of plants and the use of the microscope under botanist Franz Unger, an enthusiast for the cell theory. In the summer of 1853, Mendel returned to the monastery in Brno, and in the following year he was again given a teaching position, principally of physics, this time at the Brno high school, where he remained until elected abbot 14 years later. In 1856 he took the exam to become a certified teacher and again failed the oral part. In 1867 he replaced Napp as abbot of the monastery.

After he was elevated as abbot in 1868, his scientific work largely ended, as Mendel became occupied with his increased administrative responsibilities. The increased responsibilities prevented him from conducting any further scientific experiments. Mendel died on 6 January 1884, at the age of 61, in Brno, from chronic nephritis. After his death, the succeeding abbot burned all papers in Mendel's collection, to mark an end to the disputes over taxation.

Mendel's Genetics

In 1854 in the garden of monastery Gregor Mendel established the experimental program in hybridization . Between 1856 and 1863, he cultivated and tested some 5,000 pea plants. The aim of this program was to trace the transmission of hereditary characters in successive generations of hybrid progeny. Mendel found that garden pea (*Pisum sativum*) has many distinct varieties and he selected it to conduct his studies. Pea is suitable due to ease of growing and control of pollination and the high proportion of successful seed germinations. In period from 1854 to 1856 Mendel tested 34 pea varieties for stability of their traits. In order to understand the transmission of characters, Mendel observed seven traits that are easily recognized and apparently only occur in one of two forms: flower color is purple or white, flower position is axil or terminal, plant is tall or short, seed shape is round or wrinkled, seed color is yellow or green, pod shape is inflated or constricted and pod color is yellow or green. This observation that these traits do not show up in offspring plants with intermediate forms was critically important because the leading theory in biology at the time was that inherited traits blend from generation to generation.

He crossed varieties that differed in one trait- for instance, varieties with purple flower crossed with varieties with white flowers. The first generation of hybrid (F_1) displayed the character of one variety but not that of the other. In Mendel’s terms, one character was dominant and the other recessive. He grown numerous plants from this hybrid and obtained the second generation (F_2), in which the recessive character reappeared, and the proportion of offspring bearing the dominant to offspring bearing the recessive was very close to a 3 to 1 ratio. Study of the descendants (F_3) of the dominant group showed that one-third of them were pure line and two-thirds were of hybrid constitution. The 3:1 ratio could hence be rewritten as 1:2:1, meaning that 50% of the F_2 generation were pure line and 50% were still hybrid.

This was Mendel’s major discovery, and it was unlikely to have been made by his predecessors, since they did not grow statistically significant populations, nor did they follow the individual characters separately to establish their statistical relations.

Mendel′s knowledge in physics and mathematics, especially combinatorial mathematics served him to represent obtained results. If dominant form of a trait denote by *A* and the recessive by *a*, then the 1:2:1 ratio recalls the terms in the expansion of the binomial equation: $(A+a)^2 = A^2+2Aa+a^2$.

From these experiments, Mendel induced two generalizations which later became known as Mendel's Principles of Heredity or Mendelian inheritance. He described these principles in a two-part paper, Versuche über Pflanzen-Hybriden (Experiments on Plant Hybridization), that he read to the Natural History Society of Brno on February 8 and March 8, 1865, and which was published in 1866. Mendel's Laws of heredity are usually stated as:

1) The Law of segregation: Each inherited trait is defined by a gene pair. Parental genes are randomly separated to the sex cells so that sex cells contain only one gene of the pair. Although Mendel did not know the physical basis for heredity, he observed that organisms inherit traits via discrete units of inheritance, which are now called genes. Offspring therefore inherit one genetic allele from each parent when sex cells unite in fertilization.

2) The Law of Independent Assortment: Mendel realized that he could test his expectation that the seven traits are transmitted independently of one another. Crosses involving first two and then three of his seven traits yielded categories of offspring in proportions following the terms produced from combining two binomial equations, indicating that their transmission was independent of one another. So, genes for different traits are sorted separately from one another so that the inheritance of one trait is not dependent on the inheritance of another.

3) The Law of dominance: Therefore, a cross between a homozygous dominant and a homozygous recessive will always express the dominant phenotype, while still having a

heterozygous genotype. Recessive alleles will always be masked by dominant alleles. In a cross between two organisms pure for any pair of contrasting characters, the character that appears in the F1 generation is called dominant and the one which is not expressed is called recessive.

Mendelism - important and controversial theory

It is obviously that Mendel did not make effort to publicize his work. Today it is not known how many reprints of his paper he distributed. He had ordered 40 reprints, the where of only eight of which are known. Other than the journal that published his paper, in 19th century 15 sources are known in which Mendel′s plant hybridization work is mentioned. Few of these provide a clear picture of his achievement.

Indeed, Mendel's results were largely ignored. Although Mendel's laws were not completely unknown to biologists of that time, they were not seen as generally applicable. A major barrier to understanding their significance there was in the apparent blending of inherited traits in the overall appearance of the progeny, now known to be due to multigene interactions, while Mendel studied the organ-specific binary characters. In 1900, however, his work was "re-discovered" by three European scientists, Hugo de Vries, Carl Correns, and Erich von Tschermak but they partially still ignored Mendel's findings.

Bateson was the strongest promoter of Mendel's theory (Bateson, 1909). By 1902, he had translated Mendel's works into English and was a strong supporter of the Mendelian laws of inheritance. Bateson is credited with coining the terms "genetics," "allele", "zygote," "heterozygote" and "homozygote". Bateson first used the term "genetics" publicly at the Third International Conference on Plant Hybridization in London in 1906 (Bateson, 1906), and Johannsen first used the word "gene" in 1909 to describe the units of hereditary information. Many other biologists were against Mendel's model of heredity because it implied that heredity was discontinuous, in opposition to the apparently continuous variation observable for many traits. However, later Fisher (1936) showed that if multiple Mendelian factors were involved in the expression of an individual trait, they could produce the diverse results observed. After the rediscovery of Mendel's work, scientists tried to determine which molecules in the cell were responsible for inheritance. In 1911, Thomas Hunt Morgan argued that genes are on chromosomes and the chromosomes of cells were thought to hold the actual hereditary material, and created what is now known as classical genetics. This finally strengthen Mendel's place in history of genetics.

Discussion

The fundamental laws of inheritance are now known as Mendel's laws, and the science on which they are based is called Mendelian genetics. However, because Mendel's importance was unrecognized during his lifetime, little original information about his scientific work was preserved. Most unfortunately, his scientific records were apparently burned around the time of his death (Orel, 1996). Classical study of seven genes by Gregor Mendel was basis for modern genetics development, although relatively recently little was known about their function. Progress in molecular genetics in last few decades dramatically changed this situation and identity of Mendel′s genes in pea has been discovered. The gene Mendel studied that governs seed shape trait is *r* on chromosome 7, the gene that governs cotyledon color is *i* on chromosome 1, the gene that governs seed coat color is *a* on chromosome 1, the gene that governs pod shape is either *v* on chromosome 4 or *p* on chromosome 6, the gene that governs pod color is *gp* on chromosome 5, the gene that governs flower position is *fa* on chromosome 4 and the gene that governs stem length is *le* on chromosome 4.

Although Mendel's paper is considered a classic in the history of biology, it generated much controversy throughout the century that elapsed since the rediscovery of Mendelian laws in

1900. Some authors glorify Mendel as a brilliant scientist whose work was ahead of its time, others are critical of his methods, and a few claim he was a deception (Monaghan and Corcos, 1993; Weldon, 1902; Hartl and Fairbanks, 2007). There is substantial disagreement about his objectives, the accuracy of his presentation, the statistical validity of his data, and the relationship of his work to evolutionary theories of his day. Fairbanks and Rytting (2001) apostrophize five of the most contentiously debated issues by looking at the historical record through the view of current science: (1) Are Mendel's data too good to be true? (2) Is Mendel's description of his experiments fictitious? (3) Did Mendel articulate the laws of inheritance attributed to him? (4) Did Mendel detect but not mention linkage? (5) Did Mendel support or oppose Darwin? Some scholars disagree about Mendel's integrity in his presentation, his articulation of the fundamental laws of inheritance, his experimental design, his motives for conducting his experiments, and his conclusions. However, given the lack of suitable terminology at the time, this seems a very unpleasant judgment. Fisher (1936) certainly saw the significance of Mendel’s contribution to the field even though he was the one who raised several concerns about the data. Some of these concerns still exist, and the controversy has not been resolved, despite recent implications to the contrary (Franklin, 2008; Stigler, 2008).

Fairbanks and Rytting (2001) concluded that Mendel did not fabricate his data, his description of his experiments is literal, he articulated the laws of inheritance attributed to him insofar as was possible given the information he had, he did not detect linkage, and he neither strongly supported nor opposed Darwin.

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FEEDING THE WORLD IN 2050: FOOD AVAILABILITY VS FOOD ACCESS

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Abstract

This paper assesses the projected growth of food supply relative to population growth and estimated food demand growth over the next four decades. World population projections are analyzed for the main developed and developing regions. Implied food demand growth is then compared to grain and oilseed supply projections from a few of the most reliable sources. Three of these are ten-year projections and two extend to 2030 and 2050. To the extent possible, comparisons are made among the alternative projections. Conclusions about food availability and prices are finally drawn. Meeting the growth in demand for food, feed, and biofuels to 2050 will not be a steep hill to climb, but there will need to be continued private and public investment in technology to induce increased production growth rates through productivity enhancements and increased purchased inputs. The main food security challenge of the future, as in the present, is not insufficient production but rather increasing access and reducing vulnerability for food insecure households. The dominance of future population growth in the food insecure regions of Africa make this challenge even more critical between now and 2050 and even more so in the years beyond 2050 when climate change effects on resource constraints will be more severe.

Keywords: food supply, population growth, price projection, production growth

Introduction

In 2009, FAO issued a report (Bruinsma, 2009) that contained estimates of how much agricultural production needed to increase between 2006 and 2050 to meet the projected growing consumption from increasing population and changing diets. It was the basis for what became a widely used figure that production needed to double by 2050, even though that was only the FAO estimate for production in developing countries. The actual estimate in the 2009 report was that global agricultural production would need to increase by 70 percent. Moreover, that “double by 2050” continues to be repeated even though agricultural production has already increased substantially since 2006. More recently, FAO (Alexandratos & Bruinsma, 2012) updated these estimates and projected a 60 percent increase in agricultural production needed to provide an adequate food supply from 2006 to 2050. These figures are used later in this paper but we will first take a look at the broader context for this and other estimates of future needs.

Since the end of World War II, rapid technological change combined with inelastic demand for food has been generating declining real agricultural prices. Consumers have been the ultimate beneficiaries of agricultural innovation, while farmers have had to expand their operations and become more efficient to offset price declines. Governments in high-income countries have adopted various support and protective trade policies to shelter their farmers from price declines which often contribute to even lower market prices. This long-term decline in real prices has periodically been interrupted by price spikes caused by crop failures or other factors.

From the beginning of 2006 to the end of 2008, the world witnessed the largest surge of commodity and food prices since the early 1970s. After a short and deep price decline, they rose again between 2011 and 2014, and then began another decline in 2015 (Figure 1) The

2007/08 price surge raised the age-old Malthusian question of whether food production can keep pace with growing demand. Numerous studies have found complex factors related to this issue. Historically, the main driver of production has been technological progress. Meanwhile, the drivers of consumption have been population growth (which increases the number of mouths to feed) and income growth (which increases the quality and quantity of food consumed per person). Changing diets that accompany both increased incomes and increased urbanization generally lead to more meat consumption and thus more grain consumption per person. Aging of the population which is occurring at a faster pace in higher-income countries, may have the opposite effect on diet and quantity of grain consumed.

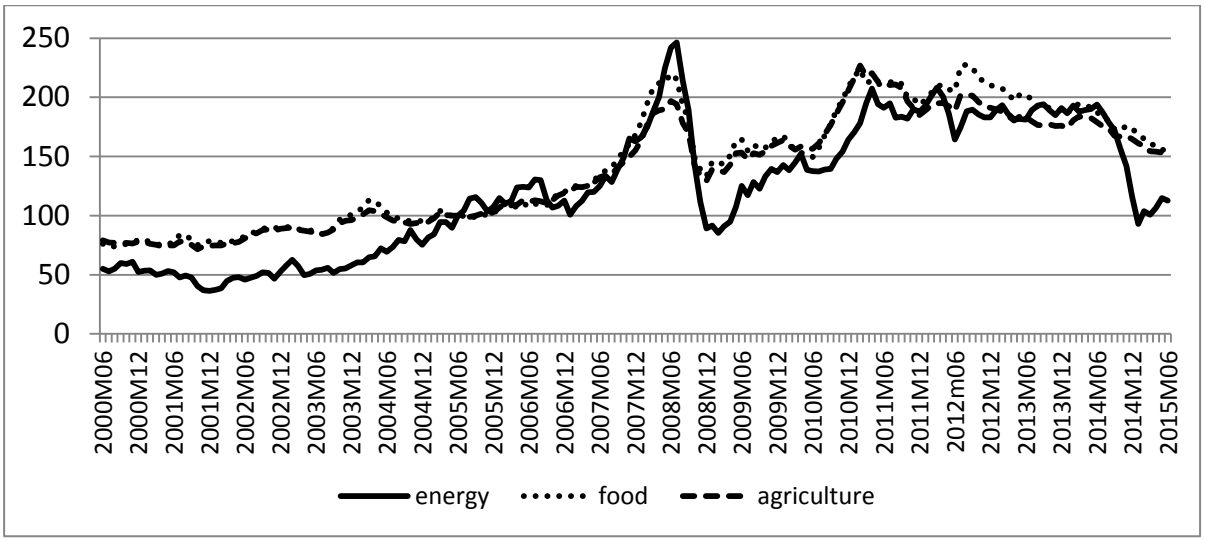

Figure 1. World Bank food, agriculture, and energy price indices, 01/00 to 6/15, 2005=100
Source: World Bank monthly commodity prices (Pink Sheet), accessed July 11, 2015.

A number of factors have contributed to the rising and more volatile prices since 2007, including declining grain and oilseed stocks, depreciation of the US dollar, poor weather (leading to yield shocks), governmental policy reaction to rising prices, changing diets, and rising demand. It has been in many ways a ‘perfect storm’ of factors (Abbott, Hurt, & Tyner, 2009; Meyers & Meyer, 2008). In a number of countries, the implementation of policies stimulating biofuel production in pursuit of environmental and farm support objectives has formed a much stronger link between fuel and food markets, a link that can contribute both to the level and the volatility of food prices (Food and Agriculture Organization of the United Nations [FAO] 2008a; Food and Agricultural Policy Research Institute [FAPRI-MU], 2008; Organisation for Economic Co-operation and Development [OECD], 2008). These policy changes have increased the profitability of investments in biofuel capacity and the increased use of existing capacity, resulting in more grains and oilseeds being used as feedstock for biofuel production. Biofuels component of grain and oilseed demand rose strongly from 2005 to 2011, but it is not expected to be a major factor in future demand (FAPRI-MU, 2015).
Examination of grain and oilseed world markets indicates that the rate of production growth was decelerating since the 1970s. This has contributed to declining stock positions in global markets (Table 1), although the new millennium saw a rebound partly in response to higher commodity prices. This rebound has continued into the second decade of the twenty-first century, but the growth rate has decelerated again. While a significant share of the decline in the 1990s was due to the restructuring and reform in the Former Soviet Union (FSU-12), even without that, grain production growth would have been slower than in earlier decades. In comparing growth rates in yield over each decade from 1960 to 2013, there has been a steady

deceleration in yield growth rates since the 1970s. Added to this problem is the finding by the Intergovernmental Panel on Climate Change (IPCC, 2007) that natural disasters may become more frequent and extreme in the future due to climate change, thus contributing to decelerating average growth in yield. Grain area which also declined between 1980 and 2000 to less than 1% per annum in production growth has rebounded, but not to previous levels.

Table 1. Average annual percent change in area, yield, and production, grains and oilseeds

	1960-70	1970-80	1980-90	1990-00	2000-10	2010-13
Grains						
Yield	2.48	1.87	1.82	1.30	1.62	1.11
Area	0.47	0.82	–0.43	–0.43	0.47	0.41
Production	2.98	2.43	1.49	0.62	2.00	1.69
Consumption	3.02	2.31	1.55	0.87	1.67	1.66
FSU-12						
Area	–02.0	0.63	–1.78	–3.03	0.07	0.14
Production	2.82	–0.06	1.15	–3.49	1.80	1.81
Consumption	3.77	2,05	0.68	–5.71	0.84	0.12
World Less FSU-12						
Area	0.62	0.85	–0.18	–0.07	0.52	0.44
Production	3.01	2.79	1.53	1.00	2.01	1.68
Consumption	2.90	2.36	1.68	1/57	1.72	1.75
Grains and Oilseeds						
Area	1.44	1.29	–0.15	0.05	0.81	0.71
Production	3.64	2.78	1.63	1.01	2.21	1.88
Consumption	3.71	2.64	1.68	1.20	1.92	1.79

Source: Calculated from PSD database, USDA (2015).

Production and consumption tend to run parallel over time. Production may temporarily outpace consumption and build buffer stocks in years of bumper crops or fast land expansion. Conversely, shrinking land use or bad weather may lead to lagging production and falling buffer stocks. Not surprisingly then, since the 1980s, consumption growth rates for both grains and total grains and oilseeds have declined, dropping below the 1% per year level in the 1990s. These, too, have since rebounded, although not to the levels seen in the 1970s and not to the same degree as growth rates in production. This suggests that decelerating population growth rates have not been completely offset by the consumption boosting effects of income growth (Alexandratos, 2008). A major factor in the consumption deceleration of the 1990s was what happened in the FSU-12, when excessive and wasteful feed use was drastically cut by market forces that rationalized grain use. Consumption growth in the rest of the world did not decline substantially in this period.

Over the past several decades, food security as an economic and political objective has grown increasingly important in all the nations across the globe. During this time the concept of food security has been changing as well - from concern for global levels of food supply, to national food self-sufficiency objectives, and more recently to a more balanced view that not only adequate national and local supplies of foods must be available but also that effective demand must be assured for the people most at risk.

We first look at population growth projections and the implications for food demand growth, and then looking at grain and oilseed supply projections from several sources. Supply and demand growth are analyzed relative to whether or not supply-demand pressures will sustain an upward pressure on prices and relative to which supply-side developments may be needed to keep pace with possible demand growth. Finally, we discuss the factors that can improve access to adequate food by those at risk of being food insecure.

Materials and Methods

First the population dynamics are taken from the US Census Bureau 2015 estimates and analyzed by region of the world. Second, the simple projection of demand is conducted with population growth, income growth projections and the income elasticity of demand for food. Then, supply projections from various alternative sources are compared to check for similarities or differences. Analysis of price projections is discussed relative to recent past price dynamics. Finally, we discuss the main factors that can improve access to food for vulnerable populations.

Population Growth Dynamics and Projections to 2050

The United States Census Bureau (2015) projects the world population to reach 9.38 billion persons by 2050, an approximate 36.5% increase over the population in 2010, while the United Nations (2012) projects 9.55 billion persons by 2050. While world population continues to increase, rates of population growth have been decreasing where incomes and education levels increase (Table 2). It is known that higher income and education levels increase the marriage age of women and reduce the number of children per family. This more than offsets increased life expectancy at birth and reduces population growth rates (Table 2). In the projected aggregate of developed countries, population begins to decline beyond 2040, and for the European continent this decline is projected to commence in 2020. Europe's birth rate has been below the replacement level for many years; only immigration has kept Europe's population increasing. In China, with its one-child policy, the population growth rate is projected to go negative in 2033 and be below the developed country level by 2050.

Table 2. Population growth rates, historical and projections in 10-year increments, 1960–2050

Region	1960–1970	1970–1980	1980–1990	1990–2000	2000–2010	2010–2020	2020–2030	2030–2040	2040–2050
World	1.82	1.66	1.58	1.29	1.10	0.96	0.78	0.62	0.48
Developing	2.20	2.00	1.90	1.53	1.28	1.11	0.91	0.72	0.56
Developed	0.89	0.68	0.51	0.39	0.32	0.25	0.14	0.04	–0.04
Africa	2.27	2.47	2.53	2.23	2.23	2.09	1.89	1.70	1.50
North America	1.48	1.28	1.12	1.21	0.93	0.80	0.67	0.50	0.38
South America	2.48	2.13	1.89	1.48	1.13	0.90	0.68	0.43	0.20
Europe	0.76	0.50	0.35	0.10	0.11	0.09	–0.06	–0.16	–0.26
Oceania	1.91	1.45	1.45	1.29	1.27	1.07	0.85	0.62	0.46
Asia	2.09	1.86	1.72	1.34	1.04	0.85	0.61	0.39	0.22
China	2.13	1.67	1.41	0.87	0.47	0.37	0.05	–0.22	–0.37
India	2.00	1.95	1.85	1.68	1.40	1.12	0.88	0.67	0.48

Source: International database, US Census Bureau (2015).

Another important aspect of global population growth is the evolution of regional distribution. From 1970 to 2010, 63% of the world population growth was in Asia and 21% in Africa (Figure 3). These numbers change dramatically between 2010 and 2050, when the total share of population growth will be about 42% in Asia and 48.5% in Africa. More than 1.2 billion people are projected to be added in Africa, which well exceeds the 1.0 billion projected for Asia. This dramatic shift in shares of population in Asia and Africa may have a significant impact on global food consumption patterns and food security because the most
food insecure regions are growing the fastest. Meanwhile, Europe's population, which has been at essentially zero growth since 1990, is expected to decrease from 11% of the world population in 2010 to 7.5% in 2050. The shares of total population in North America and South America are projected to be around 7% each in 2050. With the expected 2.67 billion

additional people between 2010 and 2050 and nearly half of these in Africa, there will be future food security challenges.

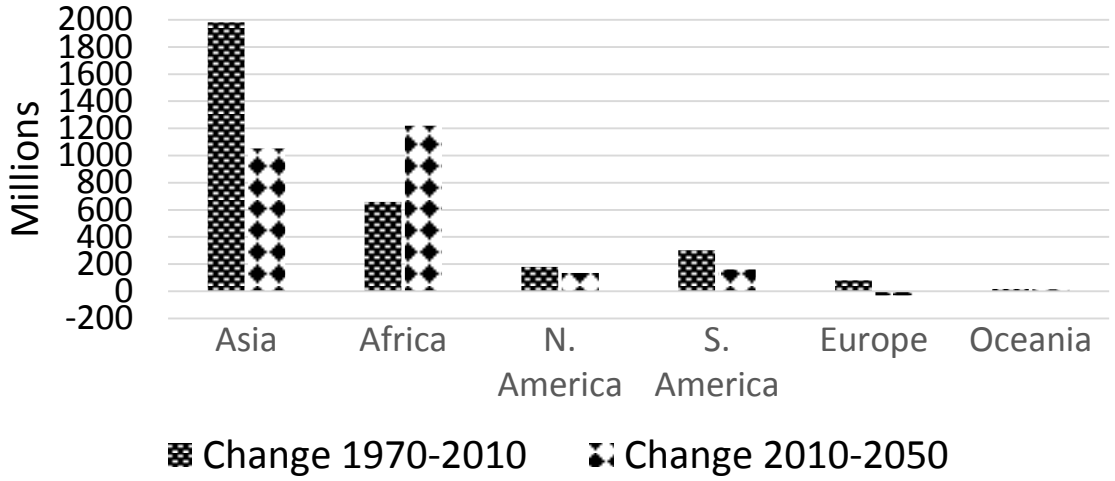

Figure 3 Regional population changes over 40 year periods past and future
Source: International database. US Census Bureau (2015).

Potential Income and Demand Growth

As an approximation of the growth in demand for food, if there were no change in per capita consumption in each country, food demand would grow at a slower rate than population simply because the populations with lower per capita consumption levels also tend to have higher population growth rates (Alexandratos, 1999). In addition, per capita consumption tends to grow with income in low-income populations, where the diet is often inadequate. At higher income levels, where there is an adequate diet, the income effect is insignificant. The percent growth in demand for food for 1% growth in income is called income elasticity of demand. We can illustrate this by comparing the calculated growth in demand for food with no change in per capita consumption (or no income effect) compared to scenarios where the income elasticity of demand for food is 0.2 or 0.4, meaning that food consumption would grow by 2% or 4% for every 10% growth in income, assuming that nothing else changes (this means there is no change in prices or other factors that may influence food demand).

Food demand growth is calculated as

Food demand growth = population growth + (income elasticity * per capita income growth)

This calculation requires a projection of the growth in income per capita for which we use the projected growth in real gross domestic product (GDP) per capita. GDP figures reflect an average growth rate of 2% or slightly higher on a per capita basis and between 3% and 3.5% per annum, including population growth. Applying the different income elasticities and using the world population growth rates from the previous section, we obtain food demand growth rates that range from about 2% per annum for the higher-income elasticity to about 1% per annum when there is no income effect or no change in per capita consumption (Figure 4). To check which of these may be closest to a recent long-term projection of food demand growth, we use the FAO (Alexandratos & Bruinsma, 2012) projection for growth rate of demand for all commodities and all uses. For the period 2000 to 2030, the estimated average growth rate is 1.4% per annum which is virtually identical to the middle calculation from 2015 onward

(Figure 4). Since population growth rates are declining, it is likely that future food demand growth will be lower.[7]

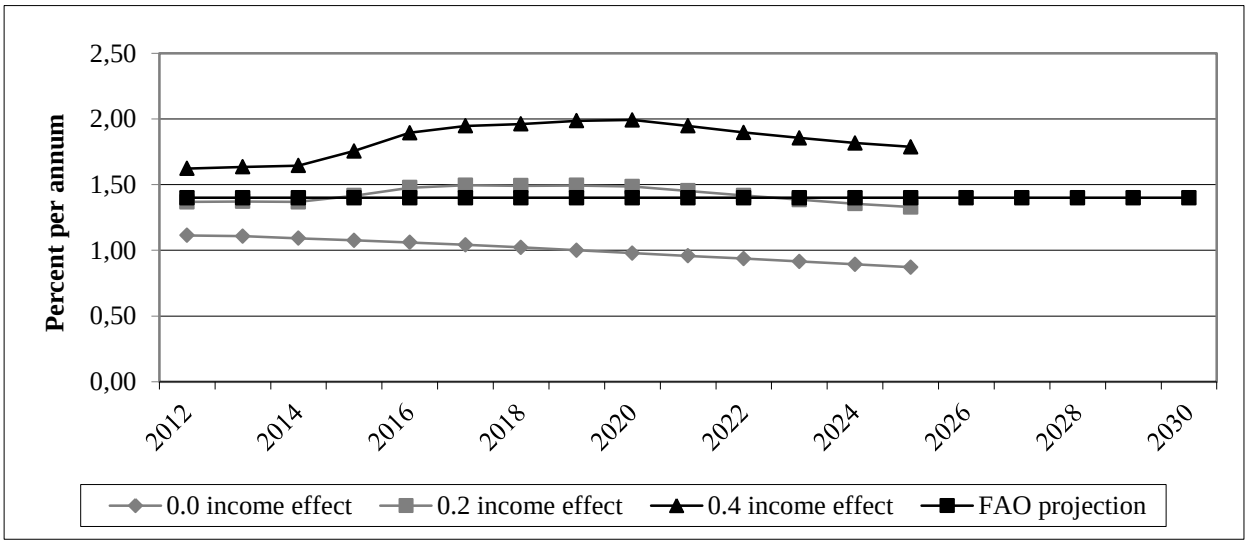

Figure 4. Calculated food demand growth rate compared with FAO long-term projections
Source: GDP growth rates are IHS Global Insight projections used in the FAPRI January 2015 baseline.

Results and Discussion

We do not want to dwell too much on food demand growth without speaking of supply and price conditions, but it is useful to have a reference point when speaking of potential growth in supply. When looking at supply growth, we focus on grains and oilseeds since they are the basic commodities from which most foods are derived. When incomes grow, people tend to shift from direct consumption of grains to indirect consumption of grain through meat (animals eat grain as a feedstuff). This is reflected in the income effect represented by the income elasticity; that is, more grain per capita is used when meat constitutes an increasing percentage of the national diet.

Supply Projections for Cereals from Different Sources

As discussed earlier, several factors have contributed to slow production growth in cereals. A key market factor was the extended period of declining real prices that reduced market incentives to invest and produce. The result was a decline in grain area (Table 1) at the same time that yield growth rates were decelerating.

Another important factor in slowing yield growth rates was the diminishing national and international public investment in agricultural research and development (R&D) that began in the 1990s. The international research investments of the 1960s were deliberate policies to enhance agricultural productivity in developing countries which resulted in the high yielding Green Revolution wheat and rice varieties that spurred yield growth and enhanced multiple cropping opportunities with shorter growing seasons. Along with continuing public and private agricultural R&D in industrial countries, improved technologies supported grain yield growth of nearly 2.5% and production growth of 3% annually from 1960 to 1970. While yield growth remained relatively high in the 1970s and 1980s, grain area declined and finally plateaued in the 1990s.

[7] This global illustration greatly oversimplifies the process used in conducting such projections. Generally, projections of demand growth must be conducted in more disaggregated ways and preferably country by country (Alexandratos, 1997).

Numerous World Bank, FAO and International Food Policy Research Institute (IFPRI) documents have established that investment in agricultural development has been lagging, especially in developing countries. Developing countries have underinvested in agriculture since the 1990s (World Bank, 2007a). Only 4% of total public R&D goes to agriculture in developing countries where 75% of the world's poor live in rural areas. Pardey, Beintema, Dehmer, and Wood (2006) find that growth in public agricultural R&D spending (which was critical to the Green Revolution) declined by more than 50% in most developing countries from 1980 onward and turned negative in high-income countries from 1991 onward. While there have been important exceptions in China and India (World Bank, 2007b), national governments and international organizations have mainly neglected these investments despite the high rates of return demonstrated in past R&D projects.

Grain and oilseed area and production have been increasing in response to a commodity price surge and increasing biofuels feedstock demand since 2006. Another contributing factor has been the steady recovery and growth of the crop sectors in the FSU-12 since 2000. It is in this context that the most recent ten-year projections of future supply have been conducted. These projections are also conducted in the context of public R&D deficiency because, even if urgent action were taken to reverse the investment path for agriculture, such action would constitute the beginning of a long-term endeavor.

The first comparison of grain and oilseed production projections comes from three well-known annual global market assessments. The first is by FAPRI-MU with assistance from the University of Arkansas on global rice market analysis (FAPRI-MU, 2015). The second is by the United States Department of Agriculture (USDA, 2015). The third is conducted jointly by the OECD and FAO (OECD/FAO, 2014). The implications of all three projections are quite similar.

Grains (except for rice) and oilseeds have had significant growth in production, primarily in response to rapidly rising prices and agricultural recovery in the FSU-12 from 2000 to 2014 (Table 3). Projected production growth rates for 2014 to 2023 are lowered, with only wheat production growth rates remaining higher than in the 1990s. Wheat is a special case in the United States because the decoupling of payments from base acres in the mid-1990s led to a large shift of land from wheat to soybeans in the 1990 to 2000 period. The comparison of FAPRI with USDA and OECD/FAO projections serves to show a considerable agreement on production growth rates.

Finally, we compare these ten-year projections to long-term projections conducted by FAO (Alexandratos & Bruinsma, 2012) and by IFPRI (Rosegrant et al., 2013). The long-term projections use different kinds of modeling systems than the ten-year projections. FAO essentially assumes constant real prices and projects that the use of grains and oilseeds for biofuels feedstock will not grow beyond 2020. IFPRI's projections also estimate prices and incorporate models of biofuel feedstock demands. The long-term estimates also take account of water and land resource constraints in the analysis. We use the FAPRI results for comparison and find a rather consistent outlook (Table 4).

Table 3. Comparison of growth rates for grains and oilseeds production, % per annum

Crop	1990–2000	2000–2014	FAPRI 2014–2023	USDA 2014–2023	OECD/FAO* 2014–2023
Rice	1.48	1.28	1.03	0.89	1.06
Wheat	0.38	1.38	0.90	0.63	0.91
Corn/Coarse grains*	1.98	3.57	1.40	1.31	1.08
Total Grains Above	1.24	2.25	1.14	1.00	1.03
Soybeans/Oilseeds*	5.25	3.98	2.24	2.38	1.57

*OECD aggregates coarse grains and oilseeds.

Sources: FAPRI (2015), OECD (2014), and USDA (2015). Historical figures use three-year average of production.

Table 4 Comparison of growth rates for grains and oilseeds production, % per annum

Crop	FAPRI 2014–2023	FAO* 2006–2030	FAO* 2006–2050	IFPRI 2010–2050
Rice	1.03	—	—	
Wheat	0.90	—	—	
Corn	1.40	—	—	
Total above/Cereals*	1.14	1.20	0.90	0.84
Soybeans	2.24	1.0	1.3	N/A
Population	1.00	1.04	0.87	0.87

*FAO and IFPRI do report aggregate cereals

Sources: FAPRI (2015), Alexandratos and Bruinsma (2012), and (Rosegrant et al., 2013).

Comparing FAO and IFPRI analyses, it is sufficient to look only at the 2010 to 2050 summary, or 2006 to 2050 for FAO, to see that they are very similar in terms of total cereals. However, the IFPRI analysis has slightly lower growth rates in grain production despite the fact that they include the growth of biofuel feedstock demand through 2050, which FAO does only through 2020. Another factor to consider when looking at these figures is that they project about a 50 percent increase in cereal production over the next 30 years, compared to a 12.5 percent actual increase over the last 4 years and 22 percent over the last 10 years.

Commodity and Food Price Projections

Projections of commodity and food prices are also important. The FAPRI, OECD/FAO, and USDA projection models include a wide range of production, consumption, trade, and price information for crops and livestock/dairy products. Price spikes in 2007/08 and shortly thereafter have opened a dialog on whether the downward path of real commodity prices has come to an end. The FAPRI price projections that accompany the most recent outlook do not answer this question. It is a price path that is neither increasing nor returning to the lowest levels seen at the beginning of the twenty-first century (Figure 4). Price projections of FAPRI include a stochastic price band that makes it clear prices will continue to be volatile whether the long-term path is increasing or decreasing. The demand for grains and oilseeds as feedstock for biofuels adds to the traditional demands for food grains and animal feeds but this growth is expected to be relatively small in the future. Declining petroleum prices have diminished these price pressures in the near term.

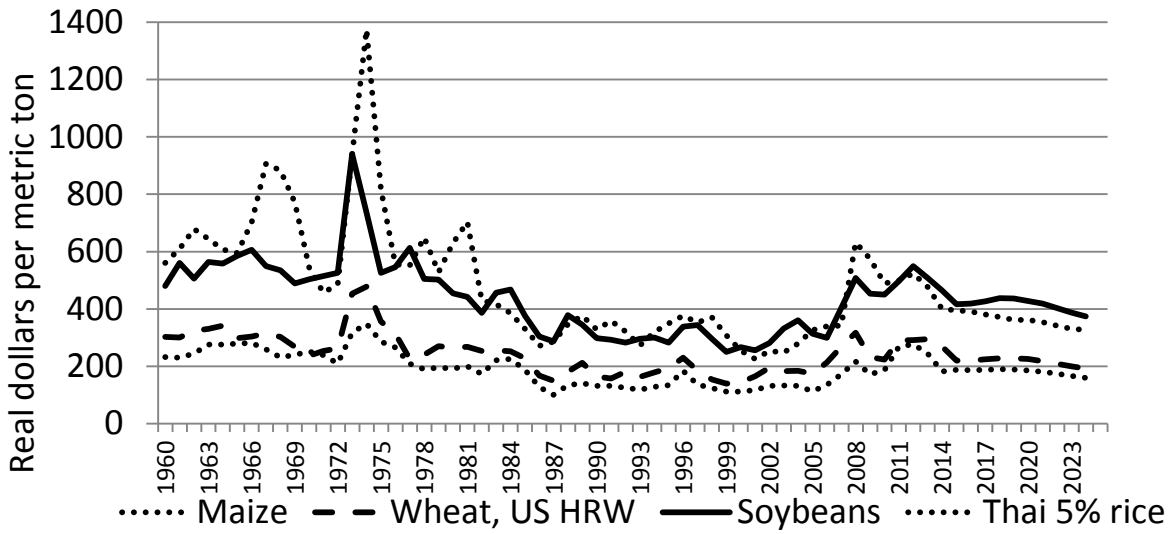

Figure 4. World grain price projections consistent with supply and demand projections
Source: History from World Bank Pink Sheet, projections calculated from FAPRI 2015 Baseline

A thoughtful recent study of alternative analyses and modeling approaches to projecting future food needs points to much research that remains to be done and alternative modeling approaches to be considered (Wise, 2013). He also highlights the post-2050 timeframe as being a much more challenging issue to analyze, especially because climate change impacts will be more pronounced. Likewise, the dominance of population growth in Africa beyond 2050 will also continue, so increasing challenges in the world's most food insecure region will be difficult. The projections cited in this paper also do not attempt to estimate the possible impacts of reducing food loss and waste, which in some locations could be a substitute for increasing production at the farm level. This is a relatively new and growing concern, and increasing information is emerging on its effects and the potential for action to reducing waste in ways that would improve food security. The questions of cost effectiveness of policies and how such actions could improve food security still need careful assessment.

Improving Access to Adequate Food

A central policy question for food-insecure regions around the world is to understand what drives changes in food security and how to incorporate this understanding into agricultural and food policy. When it comes to dealing with poverty and malnutrition, overall economic growth seems to be one of the most efficient remedies. Income redistribution and social safety net programs that target vulnerable populations are also efficient ways to address malnutrition.

A strong science and technology system contributes to innovation for equitable agricultural development and food security. Along the human history, public agricultural research and technology adoption have enabled a growing populace to avoid mass starvation. If properly focused, agricultural innovations can also enhance nutritional value of our food. Therefore, policy options boosting research and innovations in agriculture should be an intrinsic part of the national or regional food security strategies. However, the impact that public research can have on food and nutrition security is still limited since very often the scientific outputs, developed either in their own countries or elsewhere, are not easily or widely accessible to farmers. The performance of research and innovation systems can be enhanced with the help of information and communication technologies (ICTs) to improve access to knowledge in a cost-effective way, improve the demand- and technologies uptake, more effective interface

with international and national institutional partnerships and finally contribute to food and nutrition security.

Conclusions and Implications

Prior to the beginning of the twenty-first century, production and consumption growth rates have decelerated. Parallel declines in real food prices over a long period suggest that, until recently, demand pressure was not driving up prices in the food system in response to decelerating production. However, since 2000, declining stocks and a series of market shocks have led to markedly higher prices that have induced increased land use and a more rapid growth of yields and production. Limits on future agricultural land-use expansion and water resources are constraints on both production growth and consumption growth. Prices are not likely to return to the low levels seen in the early part of this millennium and will surely continue to be more volatile than in those years of stagnation. It does appear that meeting the growth in demand for food, feed, and biofuels to 2050 will not be a steep hill to climb, but there will need to be continued private and public investment in technology to induce increased production growth rates through productivity enhancements and increased purchased inputs. There will be greater land-use and water-use pressures, as well as greater pressure on food import costs, to meet food demand and provide greater food security among low-income households, especially in developing food-importing countries.

Most of these baseline projections assume a continuation of technology growth patterns of the recent past, which has seen a deceleration of yield growth rates. An alternative future would see an increased rate of technological advancement through higher private and public R&D investment which would generate a higher yield growth path, permit substitution of technology for cropland, and benefit farmers through higher productivity and consumers through lower food prices. Such an alternative higher technological future would improve the well-being of consumers, especially in developing countries, and contribute to long-term sustainability of agricultural resources by substituting technology for land, thereby reducing pressure on cropland.

The main food security challenge of the future, as in the present, is not insufficient production but rather increasing access and reducing vulnerability for food insecure households. The dominance of future population growth in the food insecure regions of Africa make this a significant challenge between now and 2050 and even more so in the years beyond 2050 when climate change effects on resource constraints will be more severe. These are also the regions where waste reduction could be a cost-effective alternative to increased production, but ideally both of these means to increase availability should be pursued simultaneously.

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HOW TO REDUCE SOIL EROSION – REQUESTS OF LEGISLATION AND EXPERIENCES WITH CONSERVATION TILLAGE IN THE CZECH REPUBLIC

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Abstract

In the Czech Republic there is more than 50% of agricultural land exposed to water erosion. It is a very urgent problem at present and mainly for the future. The goal of legislation is to protect soil against soil erosion especially on slope areas. The payment of direct support for farmers and other selected subsidies depends on fulfilment of the Statutory Management Requirements (SMR) and Good Agricultural and Environmental Conditions (DZES), which are standards of the cross compliance system. The restrictions are concerned to cultivation of wide-row crops in seriously and slightly endangered areas by erosion. In seriously endangered areas, wide-row crops (maize, sugar beet, potatoes, sunflower, bean, soybean and sorghum) are not allowed to grow. In slightly endangered areas, growing of wide-row crops is allowed, but using conservation tillage, where limits for minimum crop residue coverage were defined. Conservation tillage systems with their modification are increasingly being introduced under the economic pressure on the fields of the Czech Republic. Reducing tillage operations improves soil surface properties, including improved soil aggregation accounting for increased infiltration and percolation; less compaction due to less usage and more biological activity due to an increase in organic matter. Adding soil surface cover increases water infiltration, reducing soil drying and maintains available water for crops. Apart from above mentioned approaches in soil tillage, new crop management practices are tested with potential effect against erosion. In maize, systems based on narrower spacing and higher stand density, are evaluated in different locations of the Czech Republic.

Keywords*: soil erosion, conservation tillage, legislation rules*

Introduction

Soil erosion is a major environmental threat to the sustainability and productive capacity of agriculture. During the last 40 years, nearly one-third of the world's arable land has been lost. (Pimentel et al., 1995). Soil erosion as a physical process has been consistently studied for the last two centuries (Dotterweich, 2013) by scientists from backgrounds as diverse as geography, agronomy and engineering (Boardman et al., 2003). The use of large amounts of fertilizers, pesticides, and irrigation help offset deleterious effects of erosion but have the potential to create pollution and health problems, destroy natural habitats, and contribute to high energy consumption and unsustainable agricultural systems. It is well known, that water erosion increases on slope areas. Living and dead plant biomass left on fields reduce soil erosion and water runoff by intercepting and dissipating raindrop. Both texture and structure of soil influence its susceptibility to erosion. Soils with medium to fine structure, low organic matter content, and weak structural development have low infiltration rates and experience increased water runoff.

Soil tillage in a sustainable land management harmonises the soil protection with demands of the crop to be grown on the given land and aims soil conservation, without increasing the production risks even in the long term (Birkás et al., 2002). It can be stressed, that the demand of the crops is independent of the tillage methods. The sustainability in a soil tillage viewpoint

can evolve toward greater efficiency of resource use, and can develop and maintain a harmony between crop production technologies and soil environment (Birkás, 2013).
From above mentioned facts is visible, that crop yields on severely eroded soil are lower than those on protected soils because soil erosion reduces soil fertility and water availability.
Reliable and proven soil conservation technologies include ridge-planting, no-till cultivation, crop rotations, strip cropping, grass strips, mulches, living mulches, agroforestry, terracing, contour planting and cover crops. Although the specific processes vary, all conservation methods reduce soil erosion rates by maintaining a protective vegetative cover over the soil, which is often accompanied by a reduction in the frequency of ploughing. Each conservation method may be used separately or in combination with other erosion-control techniques (Pimentel et al, 1995). By substantially decreasing soil and nutrient loss, conservation technologies preserve the soil's fertility and enable the land to sustain higher crop yields. In many instances, the use of conservation technologies may actually increase yields.
Voluntary incentive-based measures often form parts of regional development policies. These policies have formed a basis for many cases of conservation success in Europe, including a substantial reduction in soil erosion due to a change from autumn to spring ploughing in Norway (Lundekvam et al., 2003), mitigation of harmful sheep grazing practices in Iceland (Arnalds and Barkarson, 2003), and greater farmer involvement in soil conservation schemes in Belgium (Verstraeten et al., 2003) and the Netherlands (Spaan et al., 2010).

In the Czech conditions according to the database of Research Institute for Soil and Water Conservation (2008), individual types of degradation differ with the area, the most notable being water erosion which threatens more than 50% of agricultural areas. This is followed by wind erosion (11%), extreme soils – clay soils (4.5%), and soils affected by dryness (1.5%).
In this paper we describe and analyse weak points of current Czech agriculture in relation with soil protection, introduce valid legislation principles against water erosion and present approaches of suitable soil management practices based on results from research.

Materials and methods

In paper there are presented results from different topics focused on the impact of soil tillage on soil parameters and profitable and environmentally friendly management practice for maize in conditions of South Moravia (Czech Republic).

Soil tillage and soil parameters

Grain and silage maize are crops, where acreage increased in last years. Successful soil management system is based on improving soil properties. In lowlands, especially in dry areas, water saving soil tillage technologies can be enough efficient against a lack of suitable water for maize plants during vegetation. On the other hand, the systems, which are preserving soil erosion, could be used on slope areas. The effect of different soil tillage for grain maize on chosen soil parameters was assessed in two field trials established in different soil-climatic conditions in South Moravian region. Both localities are in maize-production region. Locality Visnove is characterized with brown loamy soil, in comparison with clay-loamy fluvisol, which is in Zabcice. Average annual temperature is similar in both localities (approx. 9.0 °C), annual sum of precipitations differed (480 mm in Zabcice and 557 mm in Visnove). Different variants of soil tillage were used: CT – conventional tillage - ploughing to the depth of 0.22 m; MT – minimum tillage included soil loosening (disking) to the depth of 0.15 m and NT – no tillage (direct drilling without any tillage). In Zabcice, grain maize was grown after winter wheat and two variants of soil tillage were used (CT and MT). In Visnove, there is grain maize monoculture, all three variants were assessed (CT, MT and NT). The

grain yield was evaluated in both trials, in Visnove physical soil properties (bulk density, soil porosity, soil moisture, minimum air capacity and water infiltration into soil) were assessed as well. Kopecky's physical cylinders were taken from soil depth (0–0.10 m; 0.10–0.20 m; 0.20–0.30 m) in five replications, each year in June (2005 – 2010). A double ring infiltrometers with diameter of 0.28 m and 0.54 m in soil depth of 0.1 m were used for soil infiltrability measurement.

Effect of row spacing and stand density on silage maize yield

Maize grown in a standard row spacing (0.75 m) is connected with high risk of soil degradation by water erosion mainly on sloping plots. Except of conservation tillage technologies, growing of maize in narrow rows is approach aimed on faster canopy closure and water runoff reduction when keeping sufficient yield level. Influence of different row spacing and stand density on silage maize yield was evaluated in a field experiment in the Czech Republic. In years 2013 and 2014, a field experiment, where influence of different row spacing and stand density on silage maize yields, was established on a field experimental station in locality Zabcice (description is mentioned above). Three different row spacing were compared in both years - standard spacing 0.75 m, twin rows (rows on standard 0.75 m spacing, where each row is further divided into adjacent sub-rows spaced approximately 0.20 m) and sowing to narrow rows, which width is half of standard spacing – 0.375 m (Tab. 1). Stand density of maize was another observed factor - standard sowing rate 80.000 – 90.000 seeds per ha and end sowing rate increased about 10.000 – 20.000 seeds per ha. In 2013 these two densities were observed and in 2014 was a third sowing rate added (109.000 seeds per ha). Precise numbers of sown seeds differ in case of every technology because of different setting of drilling machine for precision sowing (KINZE 3500). Maize was sowed on 23. 4. 2013 (hybrid Silotop) and 14. 4. 2014 (Beautiful), the depth of sowing was 0.07 m. Fertilizing dose of nitrogen was 180 kg, fertilizer urea was used. Post emergent application of herbicides and insect treatment against European corn borer was made. Harvest was made in the phase of milk-wax maturity by one-row cutter in four replications.

Table 1. Overview of experimental variants in 2013 and 2014

Row spacing (m)	Technology name	Stand density (seeds per ha) – variants in 2013	Stand density (seeds per ha) – variants in 2014
0.375	narrow	1 – 85.000	1 – 87.000
		2 – 99.000	2 – 97.000
		x	3 – 109.000
0.75	standard	1 – 76.000	1 – 89.000
		2 – 80.000	2 – 97.000
		x	3 – 108.000
	twin	1 – 91.000	1 – 87.000
		2 – 102.000	2 – 97.000
		x	3 – 109.000

Results and discussion

The main characteristic of agriculture in the Czech Republic (Křen and Dušková, 2013)

Agriculture belongs to strategic branches of national economy of the Czech Republic as summarized by its production and off-production functions. Agricultural land resources occupy 54 % of the country's acreage. Production potential of the Czech agriculture represents the area of 4.25 mil. ha of farm land with more than 70 % of its plough-up. The level of arable land is higher compared to EU states with similar soil-climatic conditions. Approximately 50 % of farm land is located in less favoured areas (LFA) because of lower soil quality and adverse climatic conditions.
From a production viewpoint, basic restructuring of the sector occurred as early as the 1990's (that is prior to entry into the EU on 1.5.2004). It was accompanied by a significant decrease in production, higher unemployment and a deepening imbalance of agrarian foreign trade. After "Velvet Revolution" in 1989, in the Czech Republic, there was a period of agriculture transformation, when state farms and cooperatives were privatised. Consequently, this process had a significant impact on agronomic standards, and a lack of financial resources also negatively affected the level of inputs into the crop production.

Since entry into the EU and during gradual integration of the Czech Republic to common market and implementation of the Common Agricultural Policy, deepening of structural disequilibrium has occurred.
The efforts of enterprises are, more than to market, oriented to the subsidy obtaining which results in the following:

- Changes in crop structure, i.e. total decrease of animal husbandry, especially of pigs, decrease of acreage of crops which are too demanding on quality labour including management and marketing (fruits and vegetables), and also acreage of forage crops (clovers and alfalfa) on arable land with significant positive impacts on the environment. These structural changes cause at the same time the decrease of total agricultural production. Typical Czech farm is without animal husbandry, where "three-crops" are grown (winter wheat, winter oilseed rape and maize or spring barley).
- Although the current regulatory measures (cross compliance) and stimulation agro-environmental measures within the rural area development strive for better relationships between agriculture and the environment, further soil quality degradation, water regime deterioration and biodiversity loss continuously occur. Soil quality and water regime are predominantly affected by inappropriate large-area utilization of farm land together with reduction or even giving-up of animal husbandry accompanied with shortage of organic fertilizers. Reduction of desirable diversity of soil use e.g. by growing forage crops is also one of the courses.
- Maize (*Zea mays L.)* is a crop which has an important place in a structure of crops grown in the Czech Republic. Maize areas have been increasing all over the world. In the Czech Republic the same trend is, in spite of the fact, that during the last twenty years number of livestock, which was an important consumer of silage maize, rapidly reduced. In recent years became silage maize the main source of biomass used in biogas stations. The Czech agriculture has a free market in biomass production for energetic utilization. For this purpose, up to 900-1100 thousand ha (about 25 % of farm land) can potentially be used while maintaining food self-sufficiency. The volume of energy produced from biomass occupies within the renewable energy sources (RES) increasingly important position in the Czech mixture of energy sources.

Future development in the use of RES will be formed according to the obligations of the Czech Republic which delimitate the national goal of RES proportion of gross energetic consumption to 13.5 % till 2020 or 10 % proportion of bio-fuels in transportation. Currently, this goal is being re-evaluated at the EU level. A considerable decrease of subsidies or more strict conditions of their granting can be expected in the field of RES. Goal fulfilments will be connected with orientation to energetic utilization of agricultural commodities (including energy crops and fast-growing species) and agro-wastes and crop residues for heat production or a combine production of power and heat.

- Inappropriate use of predominantly rented farmland contributes to the increase of internal and external negative impacts of climate change on soil quality, water regime and risk to conduct a business in agriculture under more and more frequent periods of drought and floods. Climate change causes the transformation of some regions into new ones, often with adverse climatic conditions (e.g. constant increase of temperature and water shortage in the South Moravian region) together with higher importance of agriculture in less favoured areas (LFA).

Soil degradation causes annual damages estimated to be 4 to 10 billion of Czech crowns (loss of arable land and soil quality, decreased yields, clogging of watercourses, property damage etc.) and poses a significant threat for long-term competitive strength of the branch. A considerable part of waters is constantly contaminated; 82 % do not reach a suitable ecological status, and 25 % of waters do not comply with a good chemical status even though nearly no watercourses flow into the Czech Republic.
On the contrary, our country is an important European water divide and the status of ground- and surface-waters is entirely dependent on precipitations and the landscape status. Water flows very fast from the Czech area due to degradable changes in agricultural land, and thus the landscape losses its retention capacity. Great part of the present cultural landscape lost its natural characters owing to unsuitable farming. This results in lower ability of the landscape to cope with fluctuations in climate change.

National legislation rules linked with soil care, especially measures for soil protection against water erosion

Handling the negative impact of agriculture on the landscape and the environment is one of the main topics of the contemporary agricultural policy. The system of cross compliance was initiated in 2003 as part of the Common Agricultural Policy reform, and became a key element in negotiations on maintenance/evolvement of EU subsidies in agriculture in the future. The compliance with standards and requirements is ensured by monitoring the performance of so-called “control” requirements. EU member states determine their own methods of inspection on the national level. Since 1 January 2009, the system of cross-compliance is applied in the Czech Republic. The direct payments and other selected subsidies can be granted only on the condition that a beneficiary meets the statutory management requirements addressing environment, public health, the health of animals and plants, and animal welfare; the standards of good agricultural and environmental conditions (GAEC, now is used abbreviation in Czech language - DZES); and minimum requirements for fertilizer and plant protection product use as part of agro-environmental measures. In case that the potential beneficiary does not meet above-mentioned conditions, the financial support may be reduced or not provided at all.

The issue of water soil erosion is addressed mainly by the Good Agricultural and Environmental Conditions standards – DZES 4 and 5, applied to agricultural parcels listed in the Land Parcel Identification System as arable land.

DZES 4 defines soil conservation measures on arable parcels with a slope greater than 5◦. Applicants for farming subsidies on this type of land are required to sow a inter-crop after harvest, latest at September, 20th and staying till sowing of chosen spring crop or to apply one of the following measures: (1) The stubble of the harvested crop is left on the block of land or part thereof until some spring crop establishment. (2) stubble breaking and leaving the soil without ploughing till crop establishment in spring.

The main aids of DZES 5 are to protect soil against water erosion and to reduce both direct impacts of erosion and indirect impacts caused by flooding and muddy floods. There are two categories of soils classified: strongly and slightly endangered soils.
Applicants for farming subsidies (direct payments) on land classified as strongly endangered by erosion are required through cross-compliance not to grow wide-row crops on this land, i. e. maize, potatoes, beetroot, broad beans, soybean, sunflower and sorghum. Cereals and rapeseed crops are to be planted using soil conservation technologies, when crop residues cover on soil surface is at least 30 % till emergence of crop. For cereals, these measures are not required where the crop is sown into protective clover or grass-clover.
On slightly endangered soils, the applicant is required to grow wide-row crops only with soil conservation technologies. Limit for minimum crop residue cover is changing with developing of crop stand: 20% is requested during sowing, 10 % till June, 30 and visual provability of usage of conservation tillage system after July, 1.

Results from agricultural research in South Moravia

Table 2 includes the results from impact of soil tillage on soil properties. Values of bulk density increased with lower intensity of soil tillage and with soil depth. Values around 1.50 g cm^{-3} were found out in variant MT and NT (except surface layer). The highest values of soil porosity were in CT (50.24 % in average of 0-0.30 m), the lowest in NT (44.18 %). Smaller differences in soil porosity were between layers 0.10 – 0.20 m and 0.10 – 0.20 m in all variants of soil tillage. Minimum air capacity decreased with intensity of soil tillage, in MT and CT. The values in deeper layers were lower than 10 %. Soil moisture was the highest in NT, where surface and the deepest layers had the highest values. CT and MT variants had the driest layer close to the surface. Smutný et al. (2013) found out non-significant differences among variant of soil tillage.

Table 2. Physical soil properties (Visnove, 2005 – 2010)

soil layer	bulk density (g cm^{-3})			soil porosity (%)			minimum air capacity (%)			soil moisture (%)		
soil tillage	CT	MT	NT	CT	MT	NT	CT	MT	NT	CT	MT	NT
0 - 0.10 m	1.19	1.22	1.36	54.92	53.64	48.37	20.01	17.25	11.97	17.41	21.99	27.37
0.10 - 0.20 m	1.35	1.53	1.52	48.71	41.68	42.07	12.39	7.94	8.44	25.10	25.66	23.70
0.20 - 0.30 m	1.39	1.49	1.52	47.09	43.35	42.15	10.87	10.13	9.10	25.12	25.67	27.23
average 0 - 0.30 m	**1.31**	**1.41**	**1.47**	**50.24**	**46.22**	**44.18**	**14.42**	**11.77**	**9.84**	**22.54**	**24.44**	**26.10**

Above mentioned results are in relation with results of other authors. According to Birkás (2013), soil quality is focused on dynamic soil processes and properties influencing plant production risks even in the long term. Any method of tillage may be considered as beneficial

if the soil is not damaged while fulfilling plant demands or if the soil physical and biological quality is improved.

Soil tillage and water infiltration rate

The results from location Visnove shown, that the highest infiltration rate in first minutes was for conventional variant (CT) and the lowest for no tillage variant (NT). But this order was changing through time intervals (1, 10, 30 and 60 min) and the infiltration rate of NT was increasing to the level of CT variant. This corresponds to the review of Strudley et al. (2008), which describes the tendency of NT to increase macropore connectivity and deeper movement of water. Lipiec et al. (2006) noted that the differences in initial infiltration and reduction of infiltration rate with time among tillage treatments imply higher capability of conventional tillage pore system to increase amount of water infiltrating before filling macro-pores and reaching steady state. Kroulík et al. (2007) compared the differences between tillage practices at same locality in 2006. The results were similar – the highest infiltration rate was observed for CT and lowest for minimum tillage (MT) variant. Coloured water infiltration was used as well, and it showed a water saturation of CT in the top layer, while the variants with reduced tillage (MT, NT) were saturated deeper.
Results of influence of different row spacing and stand density on silage maize yields and statistical comparison of variants in both observed years 2013 and 2014 are in Fig. 1. In 2013 were statistically significant higher yields in case of narrow rows and twin rows compared to standard spacing 0.75 m. Standard technology also had lower dry matter at harvesting time. In relative values it is a difference 37 – 42 %. Only in case of twin rows stand yields increased due to an increased sowing rate (+ 28% compared to the standard sowing rate).

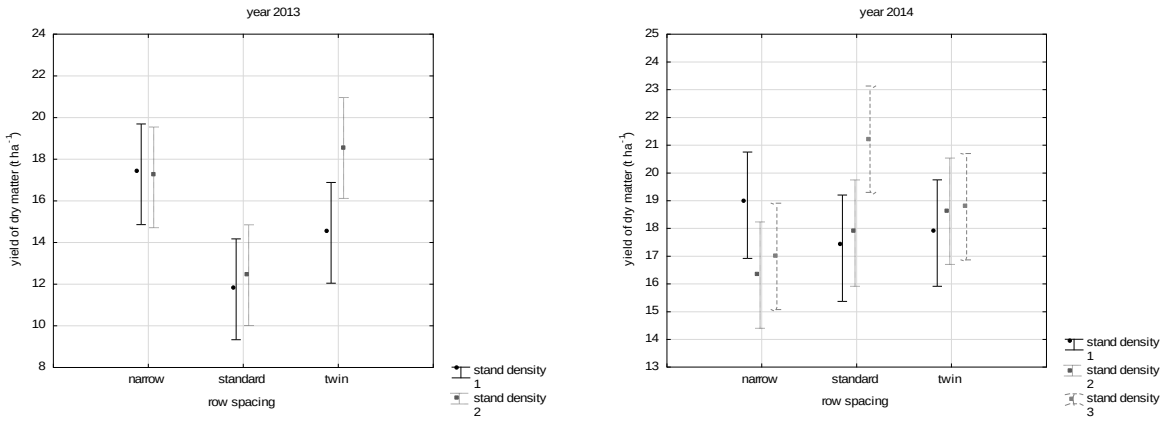

Figure 1. Yields of aboveground dry matter of maize grown under different row spacing and stand density in 2013 and 2014

Different results were reached in 2014. There were not found statistically significant differences among variants with different row spacing as in case of sowing densities. The highest yield of aboveground biomass was from variant with standard row spacing (0.75 m; 18.76 t ha^{-1}), lower from twin rows (18.39 t ha^{-1}) and the lowest from narrow rows variant (17.87 t ha^{-1}). In case of twin rows and standard technology the yields increased as sowing rate increased but the difference was not statistically significant. Twin rows proved the highest yield in case of the variant with the lowest seed rate. Explanation for different results in 2013 and 2014 can be given by different rain precipitation distribution during vegetation season. In 2013 was the first part of vegetation season humid and the summer part of vegetation season was significantly dry. Distribution of rain precipitation in the vegetation

season 2014 was in comparison to year 2013 reversed. Smutný et al. (2015) published more detailed results from this field trial, included the effect of different technologies on soil moisture, temperature and relative air humidity.

Two years results showed an advantage of maize growing in narrow rows proved by higher yields in a season with longer dry period during the first part of vegetation season. Under these conditions stand cover was involved earlier, which gave preconditions for better water management. In the wet year with sufficient amount of precipitation during vegetation season was maize yield in case of narrow rows the lowest but without statistically significance compared with others row spacing technologies. Nowadays, suitability of "narrow maize cropping system" is evaluated in the field conditions in different locations in the Czech Republic.

According to Czech legislation linked with protection of soil against erosion, different types of conservation tillage methods are applied by farmers. Conservation tillage technologies, where ploughing is replaced by tillers and shallow soil loosening, are increasingly used as soil treatments. It is typical for shallow soil tillage that all crop residues are left on the soil surface, or in the treated (tilled) upper soil layer. Crop residues can play a very important role by the next crop cultivation. Leaving crop residues on the soil surface year around, before and after planting provides soil surface protection at critical times to protect the soil against wind and water erosion. Reducing tillage operations improves soil surface properties, including improved soil aggregation accounting for increased infiltration and percolation; less compaction due to less usage of field implements; and more biological activity due to an increase in organic matter. Adding soil surface cover increases water infiltration, reducing soil drying and maintains more moisture for crop utilization. Especially conservation tillage systems with their modification are increasingly being introduced under the economic pressure on the fields of the Czech Republic (Mašek et al., 2012).

The inter crops have an important role in cropping systems. Integration of crop into crop rotation in short grow period between the two main crops protects the soil against erosion, supplies the soil with easily decomposable organic matter (Thorup-Kristensen, 1994), enhances the physical and chemical properties of soil (Eichler-Löbermann et al., 2008) and soil biological activity (Piotrowska and Wilczewski, 2012). The effect of catch crops on the reduction of erosion risk depends on the crop stand establishment, height of plants, leaf area index (LAI) and duration of soil coverage (Janeček, 2007). The highest values of coverage were found for variants with white mustard, phacelia and crambe, the lowest for buckwheat and common millet in field experiment in period 2007-2012 in Žabčice (Lukas et al., 2013). However, the results were strongly influenced by the year.

Conclusion

The results have shown that conventional and also minimum soil tillage can be suitable for maize growing, especially in lowlands. For areas endangered by water erosion, different modifications of conservation tillage are used. Suitable soil tillage management has to create good conditions for germination, emergence a development of maize stands. Conditions for high yield productivity could be in relation with improvement of soil fertility and protection soil against erosion.

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AGROECONOMIC KNOWLEDGE IN THE FUNCTION OF AGRICULTURAL EXTENSION SERVICES

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Abstract

The role of agricultural advisory services in the second half of the 20th century both in the Republic of Serbia and in other countries of former Yugoslavia consisted primarily of collecting information, innovations and knowledge from agricultural research and educational institutions and transferring them to the farmers, in order to increase production and labour productivity, and to introduce innovations and technological progress into farming practices. Advisory services mainly comprised activities related to biological, technical and technological knowledge in agriculture. The structure of employed advisers confirms this fact (only 6.6% of advisers in Serbia are agricultural economists). Development of market economy has put a focus on economic aspects of production, so agricultural advisory was faced with the need to expand its scope of work, switching to the "market-oriented" mission, which requires the introduction of an increasingly wider range of agro-economic knowledge in the activities' programme of advisory services. The European integration processes have confirmed and strengthened this tendency.

The paper analyses the state of agricultural advisory services in the Republic of Serbia and its development perspectives concerning agro-economic knowledge and activities imposed as an imperative for the current agricultural and rural development stage, as well as the preparation processes for European integration. In addition to the methodological procedures commonly used for this type of research (desk research, the method of deduction, descriptive statistics), the authors also used the results of the survey conducted in April and June 2015 on the sample of about 30% of advisers who work in Central Serbia.

The results show that most of the advisers think their scope of work has been increased in the last few years by agro-economic activities, and therefore they need some additional training on agro-economics. Since 2010 the Institute for Science Application in Agriculture (IPN), as an appointed training institution, has been constantly incorporating agro-economic courses for advisers into annual training plans. It is necessary for the Agricultural Advisory Service of Serbia to strengthen its capacities – to employ more agricultural economists and continue to organise regular training courses for the existing staff, especially in domain of agricultural economics.

Key words: *agricultural advisory, agro-economic knowledge, European integration, Serbia*

Introduction

The first written records of the agricultural advisory beginnings can be traced back to 1800 BC, to ancient Mesopotamia (modern-day Iraq), where clay tablets were found with advice on irrigation and pest management. In Egypt, data on flood advice were found. Onwards, such written records with agricultural advice can be traced from Ancient Greece and the Phoenician civilization all along to Ancient Rome (Radišić, 2015).

The beginnings of modern advisory are related to founding the first farmers' association in 1723 in Scotland, while the first practical advisory activities started in 1743. Soon after that, such associations started to emerge in other leading agricultural countries in Europe (France,

Denmark, England, Germany) as well as in the USA. In the 19th century there were eight of them, 33 more were founded by the Second World War and 49 in the post-war period (Ceranić et al., 2011).

The work of Philipp Emanuel von Fellenberg is important for advisory development in Europe. A decade after purchasing an estate in Switzerland in 1799, he started founding agricultural schools. These schools, albeit not being the first of such kind, led to spreading ideas of advisory work in Denmark, Germany, France and Great Britain. On the Universities of Cambridge and Oxford the idea of agricultural advisory gained ground in 1890s, spreading to the USA, where agricultural advisory (extension) services were established within land-grant universities. In the USA, the Morill Act at the end of 19th century allowed establishing of land-grant universities, and within them, agricultural advisory services. The Smith-Lever Act of 1914 formalised advisory tasks and defined funding models.

Further development and modernisation of the society and economy have inevitably led to emergence and growth of professional consulting services and advisory work. While agricultural advisory in developed European countries started in the mid-18th century, its beginnings in Serbia could not be traced before the mid-19th century. In the 20th century, advisory developed significantly in Europe and the USA, as well as in other parts of the world. Today it is hard to find a country without some kind of a formally organised agricultural advisory service.

The role of agricultural advisory, its form of organisation, operation and growth in Serbia and in the world have been subject-matter for numerous Serbian authors, among which: Novković (Novković et al., 1993; Somođi, Novković, 1992; Šnjegota, Novković, 1997, Novković et al., 2013), Janković S. (Janković et al., 2013; Berjan et al., 2015; Janković et al., 2015; El Bilali et al., 2015), Janković. D. (Petrović, Janković, 2010; Hoffmann et al., 2008.1; Hoffmann et al., 2008.2;), Ceranić (Ceranić et al., 2011) and others.

Agricultural Advisory Service of the Republic of Serbia

The work of the Agricultural Advisory Service of the Republic of Serbia (PSSS) is defined by the Law on Advisory and Expert Services in Agriculture, adopted in 2010 (Law on Advisory and Expert Services in Agriculture, 2010 / *Zakon o obavljanju savetodavnih i stručnih poslova u oblasti poljoprivrede, 2010*).

PSSS operates as:

- Agricultural Advisory Service in the Autonomous Province of Vojvodina and
- Agricultural Advisory Service in Central Serbia.

PSSS comprises 34 centres, covering the whole territory of Serbia and employing 259 advisers trained to provide agricultural advice. The work of 22 centres (agricultural stations and institutes) in Central Serbia is coordinated by the Institute for Science Application in Agriculture (IPN), whereas the work of 12 centres and the Oenological station – Vrsac in the AP Vojvodina is coordinated by the Provincial Secretariat for Agriculture, Water Management and Forestry. Advisory services in animal husbandry are also provided by the veterinary stations. The advisory stakeholders are also agricultural input companies, private advisers, as well as the local NGOs.

The Serbian Ministry of Agriculture and Environmental Protection together with the Provincial Secretariat for Agriculture, Water Management and Forestry provide the institutional framework for work and development of agricultural advisory services, ensuring legislation and finances. The government ensures farmers receive advice free of charge.

During the second half of the 20th century, the role of agricultural advisory services in the Republic of Serbia, as well as in other countries of former Yugoslavia, consisted primarily of collecting information, innovations and knowledge from agricultural research and educational

institutions and transferring them to farmers, in order to increase production and labour productivity, and to introduce innovations and technological progress into farming practices.
The “production-oriented“ mission also determined the content of advisory activities and services that were offered to farmers at the time. Advisory services mainly comprised activities related to the biological, technical and technological knowledge in agriculture. The structure of advisers employed in PSSS today confirms this fact (only 6.6% of advisers in Serbia are agricultural economists).
The current Law on Advisory and Expert Services in Agriculture defines advisory services in agriculture as activities performed to: “increase general knowledge and awareness of farmers; increase competitiveness and modernise agricultural production; increase profitability; enhance the quality of products; introduce the production of safe food; encourage farmers to associate; protect natural resources and the environment; improve living conditions and the culture of life in rural areas and, consequently, improve rural development“ (Law on Advisory and Expert Services in Agriculture, 2010 / *Zakon o obavljanju savetodavnih i stručnih poslova u oblasti poljoprivrede, 2010*).
Agricultural knowledge is transferred along all levels of formal education (from secondary education to PhD studies) through different types of training organised by various educational and research institution and organisations, PSSS, private enterprises, project units, media, etc. (Agriculture and Rural Development Strategy for the period 2014-2024, 2014 / *Strategija poljoprivrede i ruralnog razvoja Republike Srbije za period 2014-2024. godine, 2014*). Since 2010, advisors’ training has been organised by an authorised institution – the Institute for Science Application in Agriculture.
According to the Strategy, the general objectives of agricultural advisory services are, as follows: production of safe food, profitable production, increased competitiveness and higher quality of agricultural products, encouraging farmers to associate in interest groups, improving farmers’ knowledge, enabling farmers to run the farm successfully, protection of natural resources and the environment, and rural development. In addition to these general objectives, the Strategy also defines the following advisory activities within the scope of work of PSSS: increasing the competitiveness of agricultural production; upgrading the existing knowledge of farmers and raising their awareness; training of farmers on how to make their production and run farms more successfully; assisting farmers in production and with economic and organisational issues related to farm development; improving economic position of farms; increasing farmers’ income; adjusting production to match natural resources and market demands; encouraging entrepreneurship in agriculture and in rural areas; protecting and improving natural resources; encouraging farmers to associate in interest groups; helping farmers to plan how to improve future production and develop their farm; and rural development (Development Strategy of Agricultural Advisory in Serbia, 2009 / *Strategija razvoja poljoprivrednog savetodavstva u Srbiji, 2009*).
From the above mentioned one can see that the mission of agricultural advisory has been shifting over the last ten years from the “production-oriented“ one to the “market-oriented“ mission, so agricultural advisory was faced with the need to expand its scope of work, switching to a “market and development-oriented“ mission, which requires the introduction of an increasingly wider range of agro-economic knowledge in the work programme of advisory services. (Janković et al., 2013; Berjan et al., 2015; Janković et al., 2015). The process of European integration has confirmed and strengthen this tendency (El Bilali et al., 2015).
The authors of this paper tried to investigate whether advisers in the Republic of Serbia are ready to adjust their activities to the present requirements. In other words, whether they are ready for a new “market and development-oriented“ mission of advisory that implies a wide range of agro-economic knowledge.

Materials and Methods

In addition to the methodological procedures commonly used for this type of research (desk research, the method of deduction, descriptive statistics), the authors used the results of the survey conducted in April and June 2015 on advisors employed in the Agricultural Advisory Service of Serbia who work on the territory of Central Serbia. The survey comprised 49 advisers, which makes 18.9% of the total number of advisers employed in the Agricultural Advisory Service of Serbia (259), i.e. 29.3% advisers who work on the territory of Central Serbia (167). The survey used a questionnaire with both open-ended and close-ended questions.

Results and Discussion

The goal of the questionnaire was to show the advisers' level of agro-economic knowledge, to indicate whether there had been some changes in the structure and character of their activities in the last few years, or whether they dealt with agro-economic issues more than before. Moreover, the questionnaire also asked for advisers' opinions on whether they need some more advanced training on agro-economics. At this point, the goal of survey was not to introduce the farmers, their opinions and level of agri-economic knowledge, but there is a plan for such survey in the future.

Most of the surveyed advisers had formal education in the field of agro-economics and fruit and wine production.

Table 1. Educational profile of the surveyed advisers

Educational profile	Number of advisers	Structure (%)
Agro-economics	10	20.4
Fruit and wine production	10	20.4
Animal husbandry	8	16.3
Plant protection (Phytopharmacy)	8	16.3
Field and vegetable crop production	6	12.2
General course	4	8.1
Soil amelioration	1	2.1
Technology	1	2.1
Organic production	1	2.1
Total	49	100.0

Source: Anketa savetodavaca (Survey of advisers)

One fifth of the surveyed advisers (20.4%) were graduate agricultural economists, which implies they have a wide range of agro-economic knowledge.

Most of the surveyed advisers were young people with less than three years of experience (42.9%), or 4-6 years of experience (28.6%) in agricultural advisory.

Table 2. Year of experience of the surveyed advisers

Years of experience	Number of advisers	Structure (%)
Less than 3 years	21	42.9
4-6 years	14	28.6
7-10 years	9	18.4
over 10 years	2	4.0
No answer	3	6.1
	49	100.0

Source: Anketa savetodavaca (Survey of advisers)

When asked whether they had been required to do more agro-economic activities in their advisory work over the last 3-5 years, 75.5% of the advisers gave a positive answer, 10.2% gave a negative answer, while 14.3% did not answer at all or said they were not sure.
The advisers were also asked to assess their own knowledge of agro-economics from 1 to 5 (1 – unsatisfactory and 5 – excellent).

Table 3. The advisers' self-assessment on agro-economic knowledge

Grade	Number of advisers	Structure (%)
Excellent (5)	4	8.2
Very good (4)	7	14.3
Good (3)	32	65.3
Satisfactory (2)	6	12.2
Unsatisfactory (1)	0	0.0
	49	100.0

Source: Anketa savetodavaca (Survey of advisers)

Most of the surveyed advisers (65.3%) assessed their agro-economic knowledge as "good". Grades 4 and 5 ("very good" and "excellent") were mostly given by advisers who had formal agro-economic education.
A relatively high percentage of advisers who assessed their knowledge as "very good" and "excellent" is also a result of trainings that IPN has been continuously conducted since 2010, primarily through modules on *Farm management*, *European integrations* and other topics.
It is also interesting the advisers' assessment of previously organized agro-economic training courses. Most of the advisers (77.6%) thought the previous agro-economic training courses were not sufficient enough. Only 8.1% of the advisers considered there had been enough training courses on improving agro-economic knowledge, whereas 10.2% of the advisers thought there had not been enough training on topical agro-economic issues. Two advisers (4.1%) did not answer this question.
Finally, the advisers were also asked which agro-economic topics they need to be trained for in the future. There were offered 16 topics to the advisers where they could chose more than one item. The results are shown in Table 4.

Table 4. Agro-economic topics for future training courses

Topic	Number of answers	Structure (%)
Costs and calculations in agricultural production	20	8.5
Financing of agriculture and rural development	24	10.1
Market and prices in agriculture	19	8.0
Risks and insurance in agriculture	19	8.0
Monitoring and recording of farm data	8	3.4
FADN methodology	4	1.7
Farm management	6	2.5
Principles of cooperatives and their establishment	11	4.6
Business plans and project management	23	9.7
Agriculture and rural development strategy	9	3.8
Agricultural and rural development subsidies	8	3.4
EU pre-accession funds (IPA and IPARD funds)	41	17.3
Interest grouping (establishment of unions, associations, clusters, etc.)	17	7.2
Rural development	10	4.2
Other on-farm activities that can increase income of the family farms	17	7.2
Other topics*	1	0.4
Total	237	100.0

*Only one adviser suggested training on "Export agreements".
Source: Anketa savetodavaca (Survey of advisers)

The most of advisers (17.3%) said they need training on using the EU pre-accession funds (IPA and IPARD funds), which was expected because Serbia is about to start using money from these funds and advisers have not been trained on possibilities and applying procedures. Financing of agriculture and rural development ranked second (10.1%), and making business plans and project management ranked third (9.7%). Although the business plan making was the topic of several training courses in the past, it seems to be something that is always topical and useful.

Conclusion

Agricultural advisory has a long tradition in Serbia, where it started in the mid-19th century. Nowadays, the Agricultural Advisory Service of Serbia (PSSS) is organised through 34 centres, employing 259 advisers. PSSS is under charge of the Ministry of Agriculture and Environmental Protection and the Provincial Secretariat for Agriculture, Water Management

and Forestry (territory of the AP Vojvodina). The Ministry of Agriculture and Environmental Protection delegated the organisation of advisers' training to the Institute for Science Application in Agriculture – an appointed training institution on the territory of Central Serbia.

During the second half of the 20th century, the role of PSSS was mostly about collecting agricultural information, innovations and knowledge from research and educational institutions and transferring them to farmers, in order to increase production and labour efficiency and introduce some innovations and technological progress into farming practice. In other words, the mission of PSSS was mainly "production-oriented". The development of market economy put a focus on economic and organisational aspects of agricultural production. Hence, a wider scope of work and "market-oriented" mission has become the imperative for agricultural advisory. Since 2010 agro-economic topics have been constantly incorporated into the annual training plans for advisers' training courses organised by the Institute for Science Application in Agriculture.

In the mid-2015, the advisers employed in PSSS were subjected to a survey, by given a questionnaire. The goal of the questionnaire was to show the level of advisers' agro-economic knowledge, to indicate if there have been some changes in the structure and character of their activities in the last few years, or whether they deal with agro-economic issues more than before. Moreover, the questionnaire also asked for advisers' opinions on whether they need more advanced training on agricultural economics. The results confirmed the expected – PSSS lacks advisers with formal agro-economic education (only 6.6% of such advisers are currently employed in PSSS). A large number of the surveyed advisers (75.5%) confirmed that they had been lately required to do more agro-economic activities in their advisory work. Most of the advisers assessed their knowledge as "good" (average). However, most of the advisers (77.6%) thought the previous agro-economic training courses were not sufficient. When asked to choose among 16 of agro-economic topics for future training courses, most of the advisers (17.3%) said they need training on EU pre-accession funds (IPA and IPARD funds) most. The second-ranked topic was financing of agriculture and rural development (10.1%).

The programmes for improving PSSS should be created in such way to take into account new needs that advisory is facing, both when it comes to training programmes for currently employed advisers and training programmes for future staff. Moreover, the results of the questionnaire are also important for designing agricultural curricula of Serbian educational institutions, primarily universities, where courses in the field of agricultural economics have been recently left out from the curricula on biological and technological departments or put as the elective courses (according to the "Bologna Process" accreditation conditions). Graduate students of biological and technological departments at the faculties of agriculture in Serbia therefore today have less agro-economic knowledge, which contradicts the imperative imposed by the market-oriented economy.

It is evident that the Agricultural Advisory Service of Serbia needs to strengthen its capacities in terms of employing more agricultural economists, organising more follow-up trainings and intensifying regular training of its staff on agro-economic matters.

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PLANT PRODUCTION

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EVALUATION OF ANTIOXIDANT ACTIVITY OF TWO ALGERIAN DRY DATE PALM (Phoenix dactylifera L.) FRUITS

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Abstract

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Epidemiological studies specify that intake of fruits and vegetables have the ability to inhibit the damaging behavior of free radicals in the human body. The present research was undertaken to explore the antioxidant potential of the methanolic extract of two Algerian dry date varieties (Frezza and Mech-Degla). The research was under taken to determine the total phenolics and flavonoides using Folin-Ciocalteu's and aluminum chloride colorimetric methods. The antioxidant ability was assessed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing/antioxidant power (FRAP) and β-carotene-linoleic acid. The total phenolic content (TPC) of the two studied varieties ranged from 13.48-25.94 mg gallic acid equivalent (GAE)/100 g of fw and the total flavanoid content (TFC) ranged from 2.97-3.05 mg quercitin equivalent (QE)/100 g of fw. The DPPH assay (91.57% and 93.68%) and the FRAP assay (112.98 and 116.49 mg VitCE/100 g) showed the highest inhibition, whereas the FRAP assay (54.16 and 55.92 mg BHAE/100g ; 21.04 and 21.74 mg GAE/100 g ; 40.91 and 42.22 mg QE/100 g) and the β-carotene assay (24.19% and 24.10%) showed the lowest inhibition of methanolic extracts of two varieties Frezza and Mech-Degla, respectively. In the present research, it is found that date palm fruit extracts have remarkable antioxidant activities. Its phenolic and flavonoid contents could be responsible for its antioxidant activity.

Key words: *Date palm fruit, Total phenols, DPPH, ß-carotene and FRAP.*

Introduction

Date palm tree, *Phoenix dactylifera* L., is an important plantation crop for many countries. Worldwide production of dates in 2013 was 7 627 624.4 tons. With a production of 848 199 tons, corresponding to 11.12 % of the worldwide production, Algeria is the 4th world producer (FAOSTAT, 2013). This production ensures an important income for the national economy, providing at the same time a significant resource for local consumption.

Fruits and vegetables have been implicated in preventing or reducing the risk of coronary heart diseases, cancer and other chronic diseases. For these reasons, recommendations to increase the dietary intakes of fruits and vegetables have been suggested by many world authorities. The potential health benefits of fruits and vegetables have been partially attributed to their polyphenols contents (Abdullah Saleh et al., 2011).

These compounds are secondary metabolites that gather a large set of molecules, divided into fourteen chemical classes (Vermerris & Nicholson, 2006). They are found in all parts of higher plants (roots, stems, leaves, flowers, pollens, fruits, seeds and wood), where they have significant role in many physiological processes such as growth, reproduction and defence (Lugasi et al., 2003). These valuable molecules possess very interesting biological properties, which are used in various fields, such as medicine, pharmacy and nutrition.

In this present work we carried out a measurement of total phenolic content, total flavonoids and evaluation of their antioxidant activities (DPPH radical scavenging capacity, reduction capability and percentage inhibition of β-carotene) of methanolic extracts from two Algerian dry date varieties: Frezza and Mech-Degla.

Materials and methods

Plant material

Fresh ripe date samples used in this study consisted of two cultivars of dry dates locally known as Frezza and Mech-Degla dates. The samples were selected identically in terms of size, colour, ripening stage, without damage and calamity, and were stored in paper bags at 4 °C until use.

Chemicals and standards

Aluminum chloride, β-carotene, BHA (Butylated Hydroxyl Anisole), chloroform, DPPH (2,2-diphenyl-1-picryhydrazyl), ferric chloride, Folin-Ciocalteu's reagent, gallic acid, linoleic acid, methanol, potassium ferricyanide, quercetin, sodium carbonate, trichloroacetic acid, Tween 40, and vitamin C were purchased from Fluka Chemie (Switzerland), Merck (Germany) and Sigma-Aldrich (USA). All Chemicals and reagents used in the experiments were of analytical grade.

Moisture content

Moisture was determined according to standard AOAC method 920.151 (AOAC, 1998).

Extraction of the phenolic compounds

Ten grams of cleaned pitted fruits was extracted with 30 ml of methanol (80%) at room temperature (20°C) for 5 hours with continued agitation. After filtration and centrifugation (at 4000 g, for 10 min), the extracts were concentrated under reduced pressure at 40 °C in a rotary evaporator. The extracts were then redissolved in 10 ml of the same solvent. These concentrated extracts were used to determine total phenolics, flavonoids content, and antioxidant activities of date palm fruits. All the measurements were taken in triplicate and the mean values were calculated.

Total phenolics content

Total phenolics were estimated using Folin-Ciocalteu's reagent as described by Juntachote et al. (2007). 0.5 ml of each sample was mixed with 5 ml of distilled water and 1 ml of Folin-Ciocalteu's reagent, after 3 min, 1 ml of 7.5 % sodium carbonate was added. The final mixture was shaken and then incubated for 1 h in the dark at room temperature. The absorbance was measured at 640 nm using Shimadzu UV-120-01 spectrophotometer.

Total flavonoids content

Total flavonoids content were estimated using the colorimetric assay according to Gursoy et al. (2009). One ml of 2 % aluminium methanolic trichloride solution ($AlCl_3$) was mixed with 1 ml of the methanolic extracts. Test tubes were incubated at room temperature for 10 min and the absorbance was determined at 415 nm.

Antioxidant activities

Free radical scavenging by using DPPH radical

The DPPH radical scavenging capacity was determined using the method described by Mansouri et al. (2005). Twenty five µl of sample were added to 975 µl methanolic solution of DPPH (6×10^{-5} M) and vortexed, the mixture was left in the dark for 30 min and the absorbance measured at 515 nm.

DPPH radical scavenging capacity was estimated according to the following equation (Lu et al., 2011) :

$$\text{DPPH radical scavenging capacity (\%)} = \frac{(\text{Abs}_{515}\ \text{DPPH} - \text{Abs}_{515}\ \text{Sample})}{\text{Abs}_{515}\ \text{DPPH}} \text{ x } 100$$

Where Abs_{515} DPPH is the absorbance of the control solution (containing only DPPH), and Abs_{515} Sample is the absorbance in the presence of the date extracts.

The scavenging activity was calculated from the calibration curve. BHA (0-200 µg/ml), gallic acid (0-80 µg/ml), quercitin (0-200 µg/ml) and vitamin C (0-500 µg/ml) were used as a references to produce a standard curves. The same antioxidant capacity of the methanolic extracts of tow date cultivars was compared to those of the standards and all results were expressed as a microgram standard equivalent antioxidant capacity per gram of fresh weight (µg SEAC/g fw).

Ferric Reducing Power Assay (FRAP)

The reducing power was determined according to the method of Oyaizu (1986) with a slight modification (Gülçin, 2006). One ml of each extract was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added. 2.5 ml of this mixture was added to 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates an increase of reduction capability.

The reduction capability was calculated from the calibration curve. BHA (0-200 µg/ml), gallic acid (0-80 µg/ml), quercitin (0-100 µg/ml) and vitamin C (0-300 µg/ml) were used as a references to produce a standard curves. The reduction capability of the methanolic extracts of tow date cultivars was calculated and all results were expressed as a milligram standard equivalent per gram of fresh weight (mg SE/g fw).

β-carotene/linoleic acid bleaching assay

The total antioxidant activity was evaluated using β-carotene-linoleic acid test system (Kulisic et al., 2004 and Gursoy et al., 2009), with a little modification. Briefly, β-Carotene (2 mg) in 4 ml of chloroform was added to 25 µl of linoleic acid and 200 mg of Tween 40 emulsifier mixture. After evaporation of chloroform under vacuum at 50 °C by a rotary evaporator, 100 ml of distilled water saturated with oxygen was added by vigorous shaking to form emulsion A. 2.5 ml of this mixture were transferred into 0.5 ml of the samples. A control negative (without antioxidant) consisting of 0.5 ml of methanol and 2.5 ml of emulsion A was prepared. A second emulsion (B) consisting of 25 µl of linoleic acid, 200 mg of Tween 40 and 100 ml of distilled water saturated with oxygen was also prepared. Methanol (0.5 ml), to which 2.5 ml of emulsion B was added, was used to zero the spectrophotometer. Readings of all samples were taken immediately (t = 0) and after incubation (50°C) at 30 min intervals for 120 min at 490 nm. Measurement of absorbance was continued until the color of β-carotene disappeared.

Antioxidative activities of the extracts were compared with those of BHA (0-100 µg/ml), gallic acid (0-400 µg/ml), quercitin (0-100 µg/ml), vitamin C (0-400 µg/ml). The same antioxidative activities of the methanolic extracts of tow date cultivars was compared to those of the standards and all results were expressed as a microgram standard equivalent antioxidative activities per gram of fresh weight (µg SEAA/g fw).

The antioxidative activities (percentage inhibition) of β-carotene was calculated according to the following equation (Bourkhiss et al., 2010) :

$$AA\ (Inhibition\ \%) = \frac{A_{120\ (Sample)} - A_{120\ (Control)}}{A_{0\ (Sample)} - A_{120\ (Control)}}\ x\ 100$$

Where : $A_{120\ (Sample)}$ is the absorbance of the sample at t = 120 min, $A_{120\ (Control)}$ is the absorbance of the control at t = 120 min, $A_{0\ (Sample)}$ is the absorbance of the sample at t = 0 min and $A_{0\ (Control)}$ is the absorbance of the control at t = 0 min.

Statistical analysis

Pearson's correlation was carried out to study the relationship between the antioxidant activities (DPPH radical scavenging capacity, reduction capability and percentage inhibition of β-carotene), total phenolics and Total flavonoids. Data were reported as means ± standard deviation of triplicate experiments. Data were analyzed using SPSS statistical software (Version 21.0).

Results and discussion

Two varieties of date palm fruit from Algeria used in this study were dry dates namely Frezza and Mech-Degla (moisture content 11.51 and 15.57%, respectively).

Total phenolics content

The total phenolics content of the two cultivars ranged between 13.48 to 25.94 mg GAE/100 g fw (Table 1). Compared to other fruits, the two studied date varieties have nearly the same levels of polyphenols than those found by Dacosta (2003), in cherry, pear, peach, apricot and grapefruit which have levels varying between 60-90, 2-25, 10-150, 30-43 and 50 mg/100 g fresh weight respectively.

Dates like the other fruits are a good source of natural antioxydants and could potentially be considered as functional food (Al-Farsi et al., 2005)

Total flavonoids content

Total flavonoids of the two studied varieties Frezza and Mech-Degla are respectively 2.97 and 3.05 mg in terms of quercetin equivalent/100 g fresh weight of sample (Table 1). They account for 22 and 11.76 % of the rate of total polyphenols. Total flavonoids content of the two studied varieties are comparable with those of some fruits reported by Chun et al., (2005), who gave values of 0.63, 1.77, 5.07, 7.30 and 11.82 mg/100 g fresh weight respectively for honeydew melon, tomatoes, grapefruit, kiwifruit and oranges. Our results are slightly lower to that of some fruits reported by Lin, & Tang (2007), with a values of 14.6, 37.6 and 14.2 mg/100 g fresh weight respectively for strawberry, oriental plum and loquat.

Flavonoids are a group of polyphenolic compounds diverse in chemical structure and characteristics. They occur naturally in fruit, vegetables, nuts, seeds, flowers, and bark and are an integral part of the human diet. Dietary intake of flavonoids range between 23 mg/day estimated in The Netherlands and 170 mg/day estimated in the USA (Cook, & Samman, 1996 ; Heim, et al., 2002). Flavonoids are classified into eight groups: flavans, flavanones, isoflavanones, flavones, anthocyanidines, isoflavones, chalcones and flavonolignans. More than 8000 compounds of flavonoid structure have been identified (Hodek et al., 2002). Flavonoids are absorbed from the gastrointestinal tracts of humans and animals and are excreted either unchanged or as flavonoid metabolites in the urine and fecal slurries (Stevenson, et al., 2009 ; Crozier, et al., 2010). Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. They have been used to treat a wide variety of human conditions including inflammation, allergy, headache, cancer, viral infections, gastric and duodenal ulcers (Cook, & Samman, 1996 ; Heim, et al., 2002 ; Ndhlala et al., 2007).

Table 1. Total phenolics and total flavonoids contents of two dry Algerian date cultivars

Values are means (n = 3) ± SD ; **fw :** Fresh weight ; **GAE :** Gallic acid equivalent ; **QE :** Quercitn equivalent.

Cultivar	Frezza	Mech-Degla
Total phenolics (mg GAE/100 g fw)	13.48 ± 0.39	25.94 ± 0.8
Total flavonoids (mg QE/100 g fw)	2.97 ± 0.34	3.05 ± 0.09

Antioxidant activities

DPPH scavenging capacity of date extracts

Results of comparison between the same radical scavenging capacities of the methanolic extracts of two date cultivars and those of the standards are shown in Table 2. With a 259.42 µg/g fw for total phynolics content, Mech-Degla date showed a level of total antioxydant capacity (93.68 %) slightly higher than Frezza (91.57 %) with a BHA, gallic acid, quercitin and vitamin C equivalents antioxidant capacity values of 300.33, 97.40, 238.14 and 870.40 µg/g fw, respectively. Total antioxidant activity was also found to increase in a dose dependent manner.

Table 2. DPPH scavenging capacity and standards equivalent antioxidant capacity per gram of fresh weight of date fruits (µg SEAC/g fw).

Values are means (n = 3) ± SD ; **TP:** Total phenolics; **TF:** Total flavonoids; **DPPHSC:** DPPH scavenging capacity; **BHAEAC, GAEAC, QEAC and VitCEAC:** BHA, Gallic acid, Quercitin and Vitamin C equivalent antioxidant capacity.

Cultivar	TP (µg/g fw)	TF (µg/g fw)	DPPHSC (%)	BHAEAC (µg /g fw)	GAEAC (µg /g fw)	QEAC (µg /g fw)	VitCEAC (µg /g fw)
Frezza	134.82 ± 0.39	29.69 ± 0.34	91.57 ± 0.91	293.79 ± 4.33	95.22 ± 0.94	232.33 ± 2.49	851.83 ± 25.48
Mech-Degla	259.42 ± 0.8	30.46 ± 0.09	93.68 ± 1.56	300.33 ± 4.37	97.40 ± 0.93	238.14 ± 2.53	870.40 ± 25.86

Ferric Reducing Power Assay (FRAP)

Reducing power is one of the mechanisms of antioxidant capacity which measure the conversion of a Fe^{3+} ferricyanide complex to the ferrous form. Results of reduction capability of the methanolic extracts of tow date cultivars are shown in table 3 and all results were expressed as a milligram standard equivalent per gram of fresh weight (mg SE/g fw).

With a vitamin C standard, the methanolic extracts of Mech-Degla date showed the highest level of reduction capability (116.49 mg VitCE/100g fw) and with a gallic acid standard, the methanolic extracts of Frezza date exhibited the lowest level of reduction capability (21.04 mg GAE/100g fw).

Table 3. Reduction capability (mg SE/100 g fw) of two dry Algerian date extracts.

Values are means (n = 3) ± SD ; **SE :** Standard equivalent ; **BHAE :** BHA equivalent ; **GAE :** Gallic acid equivalent ; **QE:** Quercitin equivalent ; **VitCE:** Vitamin C equivalent.

	Reduction capability (mg SE/100 g fw)			
Cultivar	**BHAE**	**GAE**	**QE**	**VitCE**
Frezza	54.16 ± 0.26	21.04 ± 0.1	40.91 ± 0.2	112.98 ± 0.52
Mech-Degla	55.92 ± 0.28	21.74 ± 0.11	42.22 ± 0.21	116.49 ± 0.56

β-carotene/linoleic acid bleaching assay

Results of comparison between the same Antioxidative activities of the methanolic extracts of two date cultivars and those of the standards are shown in Table 4. With a 134.82 and 259.42 µg/g fw for total phynolic content, Frezza and Mech-Degla dates showed the same level of Antioxidative activities (24 %) with a BHA, gallic acid, quercitin and vitamin C equivalents antioxidant capacity values of 12, 589, 33 and 515 µg/g fw, respectively.

Table 4. Inhibition of β-carotene bleaching (%) and standard equivalent antioxidative activities per gram of fresh weight (µg SEAA/g fw).

Values are means (n = 3) ± SD ; **TP:** Total phenolics; **TF:** Total flavonoids; **IBCB:** Inhibition of β-carotene bleaching; **BHAEAA, GAEAA, QEAA and VitCEAA:** BHA, Gallic acid, Quercitin and Vitamin C equivalents antioxidative activity.

Cultivar	TP (µg/g fw)	TF (µg/g fw)	IBCB (%)	BHAEAA (µg /g fw)	GAEAA (µg /g fw)	QEAA (µg /g fw)	VitCEAA (µg /g fw)
Frezza	134.82 ± 0.39	29.69 ± 0.34	24.19 ± 3.37	12.04 ± 4.9	589.07 ± 68.06	33.66 ± 0.7	514.37 ± 7.27
Mech-Degla	259.42 ± 0.8	30.46 ± 0.09	24.10 ± 1.35	12.11 ± 4.89	589.94 ± 68.15	33.73 ± 0.7	515.10 ± 7.29

Correlation

Pearson's correlation (Table 5) shows a very high positive significant relationship between phenol and reduction capability of all standards equivalents (r = 0.966, $p < 0.01$). However, no association could be found between phenol and inhibition of β-carotene bleaching (r = -0.019, $p > 0.05$) as well as flavonoid and reduction capability of all standards equivalent (r = 0.094, $p > 0.05$). A high negative significant correlation has been observed between flavonoid and inhibition of β-carotene bleaching (r = -0.713, $p > 0.05$).

Table 5. Correlation matrix between antioxidant activities (DPPH radical scavenging capacity, reduction capability and percentage inhibition of β-carotene) and antioxidants.
TP : Total phenolics ; **FLA :** Flavonoïds ; **DPPHSC :** DPPH scavenging capacity ;
IBCB : Inhibition of β-carotene bleaching ; **RCSE :** Reduction capability of all standards equivalent ; $^{**}p < 0.01$.

	TP	FLA	DPPHSC	IBCB	RC
TP	1				
FLA	0.135	1			
DPPHSC	0.739	0.242	1		
IBCB	-0.019	-0.713	-	1	
RCSE	0.966**	0.094	-	-	1

Conclusion

The presence of compounds such as phenolics with a potential to scavenge free radicals, increase antimutagenic effects, stimulate the immune system and protect tissues from oxidative stress. Polyphenol's antioxydant defenses also have great importance in the protection of brain and nerve tissues against oxidative attacks as those causing Alzheimer's disease. The antioxidant mechanisms and different antioxidant activities should be further studied to gain more application for use as natural antioxidants. To understand their mechanism of action as bioactive components, further fractionation of methanolic extracts, isolation of phenolic compounds, and determination of their biological activities in vitro and in vivo are needed.

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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF DURUM WHEAT (Tr.durum Desf.) TO LOW TEMPERATURES

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Abstract

In the high elevation area of Algeria early genotypes of durum wheat (*Tr. Durum* Desf) suffer seriously from low temperature damage during cold season. Developing genotypes resistant to low temperature stress appear critical to ovoid crop failure and to improve grain yield. Cold tolerance is a complex mechanism linked to ecophysiological and biochemical changes.

The present study was conducted at the agricultural experimental station of National Institute of agricultural research of Algeria (INRAA). The objectives of this study were to determine the period risk of low temperature; to evaluate the genotypic variability for resistance to low temperatures through several physiological tests (crown, leaves, spike) and biochemical tests (proline and soluble sugars) and to investigate the relationships between the results of such tests, and the agronomics performances of a set of durum wheat. These tests enabled genotypes to be differentiated on the basis of their cold tolerance,

The results identified however early, freezing tolerant genotypes such as Cyprus1 and Waha which can be used as genetic source to improve tolerance to low temperatures.

Keywords: *Triticum durum* Desf., *low temperature, physiological and biochemical changes, yield.*

Introduction

In Algeria, cereal crops are practiced in a dry way; it is subjected to strong abiotic constraints (Baldy, 1974; Mekhlouf, 1998). The rainfall is low and uncertain; it is the main factor limiting the productivity of this crop (Bensedik and Benabdelli, 2000). Naturally, intermittent drought threatens the crop throughout the cycle. The other constraint is represented by low spring temperatures that reduce most often yields to their simplest expression (Laumont, 1961; Baldy, 1974; Fletcher, 1983; Abbassenne, 1998; Mekhlouf and al., 1998). This constraint leads to limit the expression of genetic potential of cultivars (Slatyer, 1974), hence, stagnation of yields (8q/ha) despite of many efforts through the intensification and support programs. Varietal selection using an empirical approach takes grain yield as a selection criterion. This approach has shown its limits, especially regarding the improvement of stress tolerance (Araus and al, 1998). For this purpose, the approach based on understanding the environment through frequency studies of the main climatic parameters, complete with phonological, morphological and physiological analysis of the plant is introduced as an alternative. This approach aims to characterize the abiotic constraints and locate it in time and to identify characteristics of tolerance to different types of stress.

The objective of this paper is to study the genetic variability of response to low temperatures and analyze the relationships with the characteristics of phenological, morphological, physiological and biochemical traits measured of several genotypes of durum wheat (*Triticum durum* Desf.).

Materials and methods

Twelve durum wheat (*Tr. durum* Desf.) genotypes were grown. The field trials were conducted from 1999 to 2003 at the agricultural experimental station of INRAA (latitude 36° 9'N and 5° 21'E, altitude 1080m). The site is representative of the high elevation area of eastern Algeria.

Grain yields, yield components and the number of days to heading were measured. The test of cold tolerance (Marshall and Kolb,1982) was carried out on the crown which was subjected to different temperatures ranging from -8 ° to -16 ° C with step of -2 ° C. The same test on spike at levels of temperatures of -3°C to -5°C was used. The effect of cold was evaluated on a scale of 1 to 3 (Mekhlouf et al., 2001). Proline and soluble sugars were measured during two seasons of experimentation on vegetation samples taken from the genotypes planted in field at tillering stage (Bridger et al., 1995).

Data analyses was performed using the statistical software Irristat version 5 yield and its components were analysed according to a factorial experiment conducted in a completely randomized block design with three replication. Least Significant difference (LSD), at 5% was used for means separation. Principal component analysis was performed on the basis of the correlation matrix among the measured variables

Results and Discussion

Frequency of climate and risks: freezing and water deficit

Figures 1 and 2 highlight the levels of risk on cereals sown each year in semi-arid area. The water deficit is beginning to appear as early as February and becomes more intense as the plant grows (Figure1). Figure 2 highlights the presence of late frosts in spring coinciding with the stage of high sensitivity of the crop. The varieties that spikes emerged during April are in danger of freezing; the effect is measurable on the number of kernels per spike.

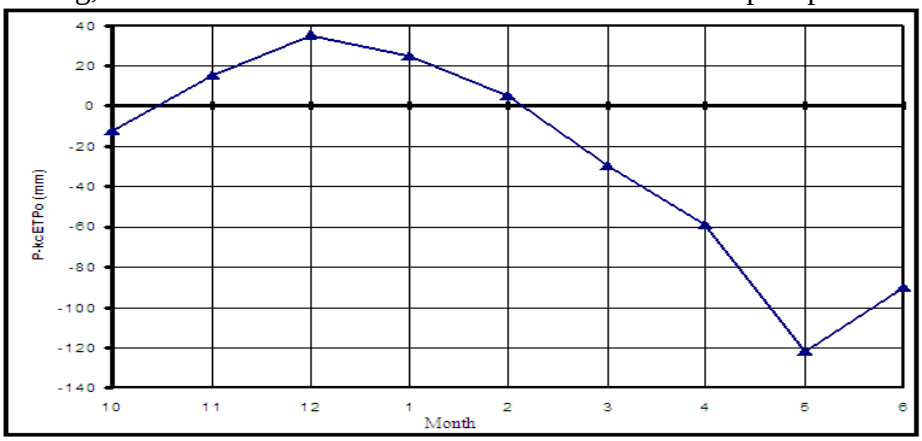


Figure 1. Variation of the average rain deficit for the experimental site over the period 1980/2000

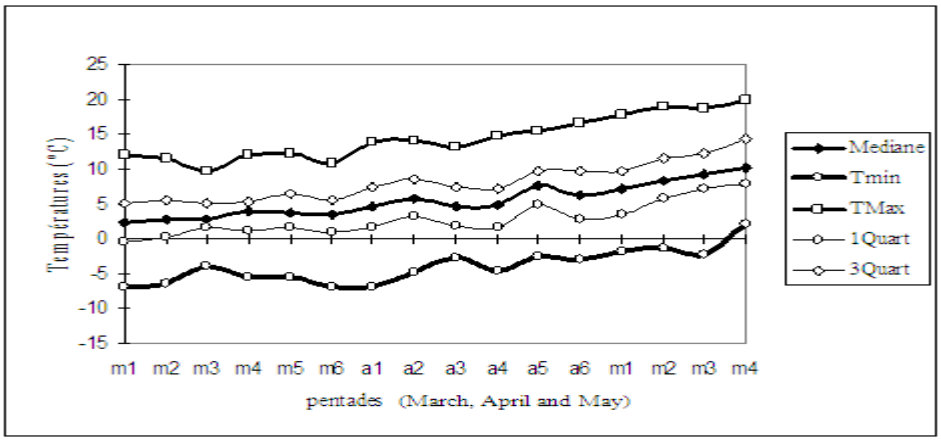


Figure 2.Frequency of the minimal temperatures, pentad of sensitive phase of durum wheat.

Environmental effect on the expression of the variables

The year effect is by far the most important, explaining much of the observed variation in the variables analyzed (Table.1). This effect highlights the high variability of growing conditions which is subject of durum wheat in the highlands. The genotype effect is significant only for the duration of the vegetative phase and harvest index (Table 1). The ranking of genotypes for these two variables remains relatively constant from one year to another, showing little interaction.

Table 1. Average squares of variance analysis of the variables measured during 4 years.

Source of Variation	Df	PVG (D)	HT (cm)	NE (/m^2)	PMG (g)	BIO (100kg/ha	NGE	GY (100kg/ha)	HI (%)
year (A)	3	859*	1594*	961666*	1019*	27347*	3056*	3574*	222.6*
Blocs/year	8	7.2	56.3	1725	3.7	125	8.7	23.5	7.3
Genotype (G)	11	199*	522ns	21888ns	32ns	979ns	48ns	217ns	173.9*
G x A	33	28*	286*	18956*	30*	1580*	103*	216*	64.8*
Error(/Residus)	88	12.2	78.2	3543.2	6.3	156.6	14.4	30.1	18.6

PVG= vegetative phase, HT= straw height, NE = spike per m², PMG = 1000 grain weight, BIO= dry matter or biomass, NGE= seed number/spike, GY= grain yield, HI= harvest index; Df= degrees of freedom, D=day
ns, *,** = not significant and significant effect at 5 and 1 % respectively.

The change in growth conditions between the different seasons induced very different genotypic responses for the remaining variables. This environmental variation leads to a change in ranking of genotypes between years. 1999/2000 and 2000/2001 seasons have been less favourable to the expression of grain yield, with an average of 3.29t/ha. Both following seasons have been relatively more favourable; with an average yield of 5.01t/ha (Table 2). The difference of 1.72t/ha between the average performance of favourable and unfavourable seasons was significant. It is associated with a long duration of vegetative phase, a higher biomass and yield components taking values over the average of four seasons of study (Table 2). Quite similar yields are obtained in the two unfavourable seasons through very different performance development itinerary. These results confirm those of Ceccarelli and al. (1991) and Bahlouli and al. (2004) who find that the same level of grain yield is obtained in different combinations of characters.

Thus, the 1999-2000 season allowed the expression of the number of spike per m^2 but unfavourable to the number of kernels per spike, whereas the contrary is recorded in 2000/2001. The differences in biomass, the number of days to heading, thousand kernel

weight and harvest index are not significant between these two years (Table 2). Kirby and al. (1992) mentioned the phenomenon of compensation that is set up between the components of yield. The number of spikes per m^2 is formed early in the beginning of the cycle, once the main strand begins its elongation immediately after the completion of meiosis stage (Hoshino and Tahir, 1987). This component is determined once the swelling stage is achieved. Winter water stress and / or low temperatures of early spring can cause a significant reduction in this component (Gate, 1995). The number of kernels per spike began its formation just before heading, at the meiosis stage, and ends 10 days after heading (Wardlaw, 2002). It is very sensitive to low spring temperatures (Bouzerzour and Benmahammed, 1994, Mekhlouf and al. 2006). Water stress and high temperatures for the period of 10 days before and after the date of achieving the heading stage are also deleterious to this component (Moncor and Wardlaw, 1995).

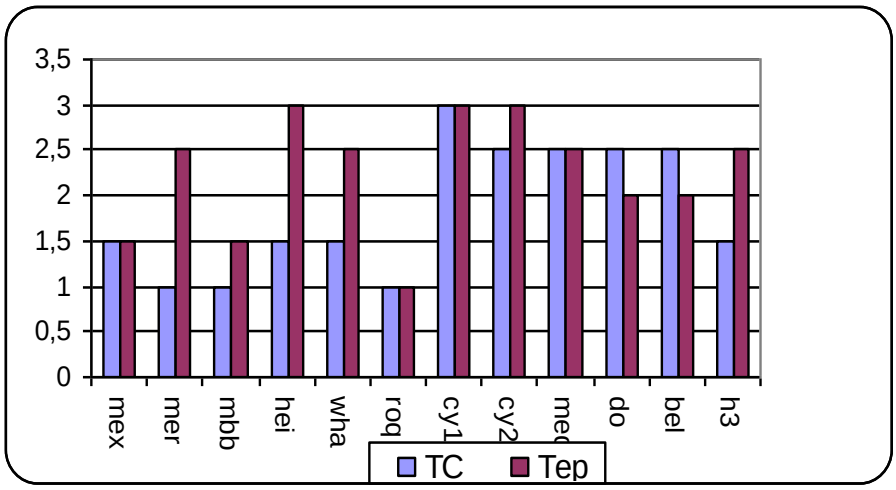
Table 2.Season and genotypic main effects of the measured variables

	PVG (d)	HT (cm)	NE (/m^2)	PMG (g)	BIO (100kg/ha)	NGE …	GY (100kg/ha)	HI (%)
Season main effect								
1999/00	117	64	649	37.1	69	15.4	33.8	48.6
2000/01	119	67	278	36.8	65	30.4	31.9	49.3
2001/02	128	63	459	33.9	117	32.9	50.5	43.9
2002/03	121	78	340	46.2	112	36.3	49.7	45.8
Genotypic main effect								
Mexicali75	117	62	481	38.7	98	29.4	47.0	50.6
Merouani	125	70	471	40.2	88	28.0	40.3	46.3
MBB	127	65	463	40.3	87	24.4	39.9	44.9
Heider/Martes//Huevos	119	62	387	38.0	89	31.2	44.6	50.2
Waha	116	66	427	35.2	84	27.2	41.4	49.8
Roqueno	118	70	453	40.6	110	27.9	46.9	45.7
Cyprus1	117	66	428	36.3	83	30.0	41.8	50.9
Cyprus2	119	71	464	37.6	99	31.4	47.5	49.5
Medolla	123	68	362	38.3	86	27.4	36.9	42.7
Durum d’Oran	127	74	455	38.3	99	30.3	36.6	39.8
Beliouni3258	124	61	435	39.8	79	27.7	40.0	50.3
Hedba3	124	85	354	38.7	84	30.0	34.9	42.5
General average	121.7	68.5	432	38.5	90.9	28.7	41.5	46.9
LSD 5%	3.7..	..	..	2.5..	..	5.8..	32.2..	..

PVG = number of days to heading, HT = straw height , NE= number of spike per m², PMG= thousand Kernel weight, BIO = biomass, NGE = number of kernels per spike, GY= grain yield , HI = harvest index (%), d=day.LSD little significance difference

Responses to low temperatures

The cold test performed on the crown showed Cyprus1, Cyprus2, Durum d’Oran, Medola and Beliouni3258 as tolerant and Merouani, MBB, and hedba3, Roquéno as sensitive (figure 3). The test performed on spike confirms the tolerance of genotypes Cyprus1, Cyprus2, Heider //Martes/ /Huevos de oro (Figure 3).



TC: crown test, Tep : spike test

Figure 3: Response of the genotypes to the low temperatures.

The capacity of accumulation of proline and soluble sugars varies according to seasons and genotypes. The differential ability of accumulation of these substances in low temperatures is taken as a measure of tolerance of the genotype (Heber and al., 1971; Heller and Green,1981). Average of two seasons, Mexicali, Medolla and Durum d'Oran accumulate more proline, while Heider/Martes//Huevos de oro, Roqueno and Cyprus1 accumulate more soluble sugars (Figure 4).

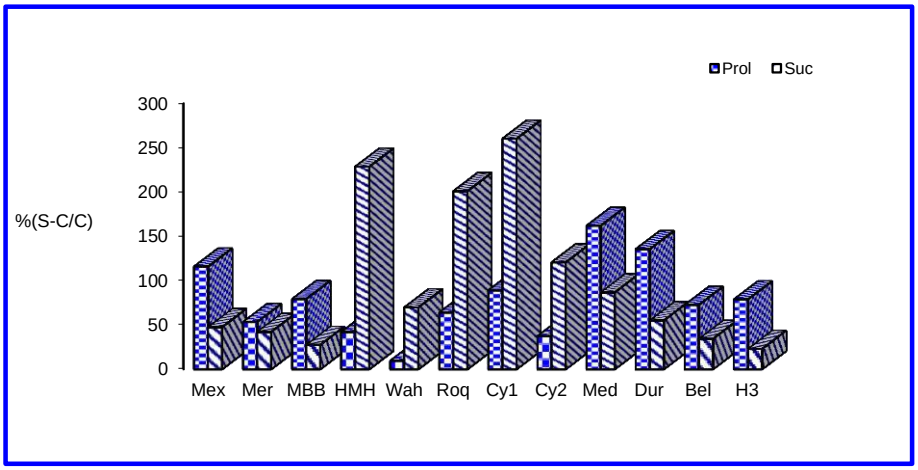


Figure 4. Capacity of accumulation, under low temperatures, of proline and soluble sugars 100 [(Stress-Control)/Control], mean of 2 seasons

H_3 = Hedba$_3$, MBB = Mohammed Ben Bachir, Dur = Durum D'Oran, Med= Medolla, Mer= Marouani, Cy_1= Cyprus1, Cy_2= Cyprus2, Wah= Waha, HMH= Heider/Martes//Huevos de Oro, Mex= Mexicali$_{75}$, Roq= Roqueno, Bel= Beliouni$_{3258}$).

Results revealed also that in the stressful conditions, genotypes Cyprus1, Heider/Martes // Huevos de oro and Medolla were high cold tolerance. This tolerance is confirmed by the level of accumulation of proline and sugars. This ability to accumulate these substances as a result of heat stress appears to be related to cold tolerance and has been noted by several authors (Santarius, 1973; Line Berger and Steponkus, 1980, Heber et al, 1971), who note that cell destruction can be prevented by the accumulation of these substances. In parallel, the genotypes Roqueno presents conflicting results. Indeed, this variety showed significant levels of soluble sugars and proline and sensitivity to low temperatures according to various tests of cold used (figure 3 and 4). Results showed also that some genotypes such as Cyprus1,

Cyprus2 and Heider/Martes//Huevos de oro present both an adaptation to cold through physiological tests and a significant accumulation of proline and soluble sugars in samples stressed. These results confirm those obtained by Havaux and Lannoye (1982), indicating that the accumulation of free proline is more marked as the plant is more tolerant to cold.
The principal component analysis (PCA) accounted for 74.1% of the variation. PCA1 accounted 33.4% of the variation and represented number of days to heading, the number of kernels per spike, harvest index, biomass and straw height toward its positive direction (Figure 5). And spike cold test, thousand kernel weight variance and harvest index toward its negative direction. PCA1 represented the overall variation between seasons for all variables measured except grain yield (Figure 5). PCA2 represented the number of days to heading, the number of spike per m², grain yield, biomass and differences among soluble sugars measured during seasons 2001 and 2002. Along PCA2 axis, are opposed phenology of production capacities and accumulation of soluble sugars and proline., in this case grain yield, biomass and soluble sugars. According to their position along the axis PCA1 and PCA2: along PCA1 genotypes Heider // Mt // Ho, Waha and Cyprus1 had a tolerance to late cold, low and varying thousand kernels weight and low across season straw height, the number days to heading, the biomass, the number of kernels per spike and harvest index. Along the same axis, genotypes Roquéno and durum d'Oran showed the opposite characteristics (Figure 5). They had below average thousand kernels weight, are sensitive to cold late; a low across season variance thousand kernels weight but high thousand kernels weight, high across season variances for straw height, biomass, harvest index, number of kernels per spike and number days to heading (Figure 5). The genotype Durum d'Oran (PCA2,) is less productive. Heider / Mt // Ho, Waha and Cyprus1 have an intermediate productivity. Roqueno is the most productive genotype. Along axis 2, local genotypes Medolla, Durum d'Oran and Beliouni$_{3852}$ oppose varieties introduced Mexicalli75 and Cyprus2. Local genotypes are characterised by a high number of days to heading, low number of spikes per m², low grain yield, a significant biomass production and low accumulation capacity of soluble sugars. In contrast, the introduced genotypes are characterised by an early heading and high number of spikes per m², high grain yield and , biomass but a low accumulation of soluble sugars under stress.

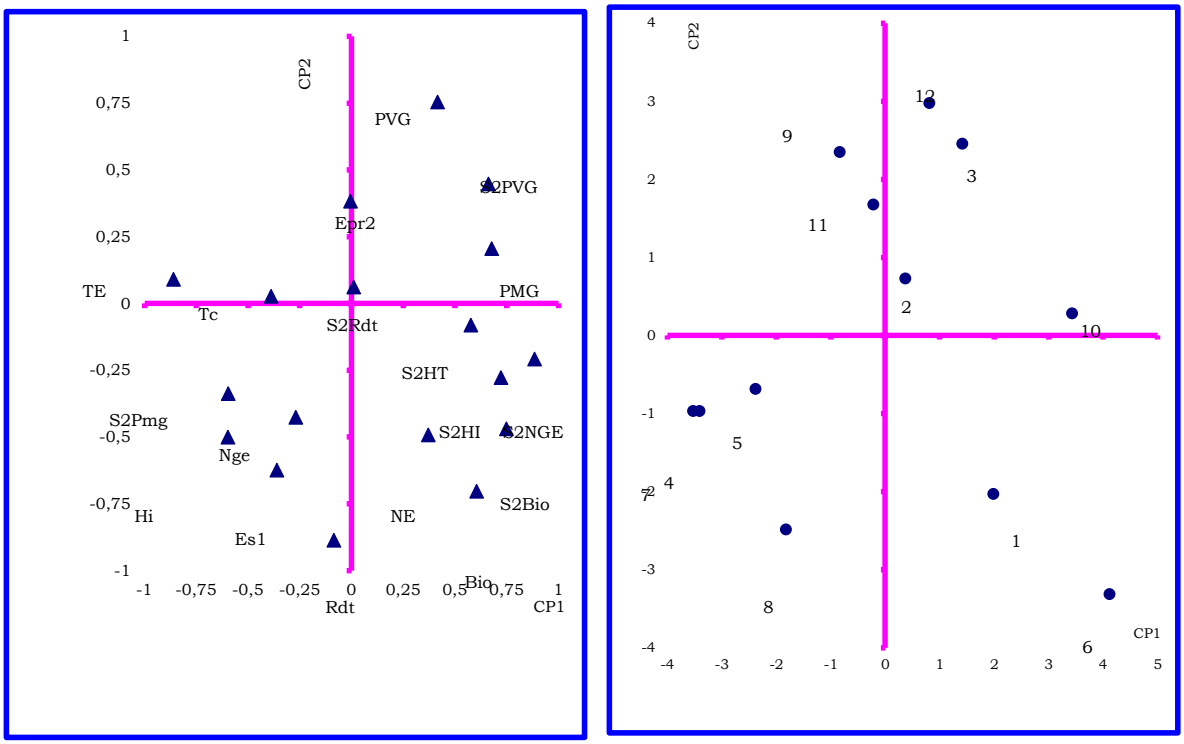


Figure 5. Principal Component Analyses (PCA) projections on axes 1 and 2

PVG = number of days to heading , HT = straw height, NE= number of spike per m², Pmg= 1000 kernel weight, BIO = biomass, NGE = Number of kernels per spike, RDT= grain yield , HI = harvest index. Tc=crown test 1=Méxicalli$_{75,}$ 2=Merouani, 3=Mohammed Ben Bachir, 5=Waha, 6=Roqueno,7=Cyprus$_{1,}$8Cyprus$_{2,}$9=Medolla, 10= Durum d'Oran, 11=Beliouni$_{3258}$ 12=Hedba$_3$ 4=Heider/Martes//Huevos de Oro

Conclusion

In the high elevation area of eastern Algeria, durum wheat suffer seriously from environmental stress factors that can prevent the expression of the genetic potential . The results of the present study showed that tests used were effective in sorting out the genotypes which were low temperatures tolerant those wich were sensitive. The measured agronomic traits varied between seasons and genotypes indicating the presence of significant genotypic variability and differential responses to the growth conditions experienced. The results identified however early, freezing tolerant genotypes such as Cyprus1 and Waha which can be used as genetic source to improve tolerance to low temperatures.

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EFFECT OF REGULATED DEFICIT IRRIGATION ON GROWTH AND WATER REGIME OF POTATO

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Abstract

The aim of this study was to determine the effect of regulated deficit irrigation (RDI) on potato growth and plant water regime in the field conditions and to compare these effects with the conventional method of irrigation. The field potato (*Solanum tuberosum* L. cv Liseta) experiments were conducted during 2007. and 2008. In 2007. a static approach in regulated deficit irrigation technique was applied and the plants were irrigated with 70% of water compared to plants that are optimally irrigated (FI) during the whole season, while a dynamic approach was applied in 2008. where the amount of water for irrigation was reduced from 70% to 50% during the last 3 weeks of the irrigation period. During these two seasons, the following parameters were measured: water potential and stomatal conductance, plant height, leaf area and leaf area index (LAI). The results showed that in the both seasons the regulated deficit irrigation didn't significantly affect the plant growth parameters as plant height, leaf area and LAI compared with optimally irrigated plants, but the differences were expressed between individual harvests. Analysis of the plant water regime parameters point to the differences between two seasons in a way that in 2007. the chemical signals were more responsible for the reduction of stomatal conductance, while in a 2008. season the reduction of stomatal conductance was followed by a decrease of leaf water potential that indicated the hydraulic drought signals.

Key words: *regulated deficit irrigation (RDI), potato, plant growth, water regime*

Introduction

Agriculture is highly sensitive to climate change and especially, to drought. The increase in temperature can increase duration of the crop growing season in regions with a relatively cool spring and shortened the season in regions where high summer temperature already limits production. The clean freshwater becoming a limited resource and its use for crop irrigation is in competition with the demand for household consumption, as well as with the need to protect the aquatic ecosystems. It is obvious that saving clean water, increasing agricultural productivity per unit of water (“more crop per drop”) and producing safe food are becoming of strategic importance for many countries (Luquet et al., 2005).The reduction of yield in drought conditions is most likely due to the decrease of growth, but also as a result of shortening the growing season (Samara et al., 2005). In drought conditions shoot growth inhibition is associated with the closure of stomata (Holbrook et al., 2002), which caused a decrease in transpiration, conservation and plant water use efficiency (Tahi et al., 2007). In Serbia and countries in the region, drought is also a significant stress factor, and its negative impact particularly affects the breeding and productivity of crops, especially vegetables, including potatoes.Potato is an important crop worldwide. The crop is very susceptible to water stress and hence irrigation is needed for achieving acceptable yield and quality (Liu et al., 2006).The potato plants have high demands for water and irrigation is necessary for a successful growth (Fabeiro, 2001). In the last decades, one of the management options to overcome the agricultural drought is the use regulated deficit irrigation (RDI) and they are called deficit irrigation method. DI have been extensively and successfully tested on many

field and horticultural crops without significant loss of economic yield (Sepaskhah and Ahmadi, 2010). RDI method is based on the understanding of the physiological responses of plants to water supply and water deficit, especially the perception and transduction of root-to-shoot drought signals (Chaves et al., 2002; Morison et al., 2008). Regulated deficit irrigation (RDI/DI) is a method that irrigates the entire root zone with an amount of water less than the potential evapotranspiration during whole or specific periods of the crop cycle (English & Raja, 1996). The principle of the RDI technique is that plant sensitivity to drought is not constant during the growing season and that intermittent water deficit during specific periods of ontogenesis may increase water savings and improve yield quality (Loveys et al., 2004). When the roots are in contact with dry land becomes a source of chemical signals ABA primarily as inhibitors of growth. The increased content of ABA leads to partial closure of stomata, which causes the reduction of transpiration and leaf growth, but also increase plants water use efficiency (Davies et al., 2002). Whole plant growth is usually limited by dry matter production and leaf area expansion and, therefore**,** traditional plant productivity analysis is based on the investigation of several biomass and assimilatory area parameters

Materials and methods

Experimental design and cultivation

The experiments were carried out during the growing seasons of 2007 and 2008 in a vegetable commercial farm (“Salate Centre”), located 10km north of Serbian capital, Belgrade. The soil of the field was silty-clay and it was developed on alluvial deposit.

Potato (*S. tuberosum* L.) cultivar Liseta was used for investigation.The field was organized as split plot design with six plots (three per treatment). The seed tubers were planted in the beginning of April at the depth of 10 cm, with distance between plants in a row of 30cm and distance between rows of 75 cm. Seed tubers were ridged with 15cm soil and ridges were formed about 30 days after planting. During the vegetation season plants were treated against weeds and fungal disease. Fertilization included an initial application of nutrients (N, P, K and micronutrients).

Irrigation scheduling and treatments

Irrigation amount was controlled by programmable irrigation equipment that supplied their irrigation through a drip line system to each plot.The irrigation method used was a drip-subsurface method. The subsurface irrigation system was supplied by Netafim (A.C.S. Ltd.Netafim, Israel). For the FI and RDI treatments, one drip line was placed 10cm below the top of the ridge. The distance between emitters in FI and RDI treatments was 30 cm and these were place exactly in the middle between two plants.With the RDI treatment is started after the phase of tuber initiation (when 80% of tubers more than 20 mm long). In the last 3 weeks of the irrigation period, 70% RDI was replaced by 50% (RDI plants received 50% of FI treatment). Irrigation was done at least twice per weekand done with a time domain reflectometer (TDR, TRASE, Soil Moisture Equipment Corp.,USA) using vertically installed probes.

Experimental measurements

The leaf water potential (Ψl) was measured using the pressure chamber (Scholander et al., 1965). The method is based on the extraction xylem sap from the leaves under the pressure of the gas usually nitrogen.Stomatal conductivity was measured using diffusion porometer AP4 (Delta-T Devices). During the experimental period we measured the: water potential and stomatal conductance, plant height, leaf area and leaf area index (LAI). Leaf area was determinate destructively by sampling leaves from plants and than scanned by scanner

(Mustec Scan Express A3 USB). Leaf area index (LAI) is the ratio of total leaf area of the plant to the soil surface.The measured traits have been analyzed for statistically significant differences by Students unpaired t - test (Sigma Plot 6.0 for Windows – SPW 6.0, Jandel Scientific, Erckhart, Germany)

Results and Discussion

Leaf water potential (Ψl) and stomatal conductance (g_s)

Fig 1. shows the changes in leaf water potential and stomatal conductivity of plants under the influence of FI and RDI irrigation regimes in 2007. year. Between harvest the values of stomatal conductivity were decreased by 68% in FI and RDI regime. The values of stomatal conductivity with FI and RDI irrigation regimes at the end of the experiment were: 0,19 and 0,21 mol m^{-2} s^{-1}. Maximum values of water resources leaves were recorded for FI irrigation regimes in phenological stage when tuber reached 50% of its total mass, while harvesting H3, and the RDI maximum values were at the stage of flowering plants during the harvest H1. For FI, RDI maximum values were: -0.82 and -0.97MPa. Decrease the values in water potential was recorded with FI irrigation regimes between harvests H3 and H4 in phenological development phase where tuber reached 50-60% of their final mass. Values water potential at the end of the experiment were the FI -1.12 MPa, RDI-1.0 MPa. In the period in 2008. (Fig.2) were similar results as in 2007. The decrease in water potential and conductivity of the stomata was consistent in all irrigation regimes. The results obtained showed that RDI the plants at the end of the experiment had a higher level of stress. The decrease in water potential RDI plants indicates that the water stress in the zone of the root system was sufficient to cause reduction of water potential of leaves. Our results are in agreement with the results Wakra et al., (2004) who examined the beans in water resources affected by RDI irrigation regimes where there has been a decrease in the value of water resources in relation to the control. Similar results in studies of effects RDI irrigation regimes on tomato plants were given by Topcu et al., (2006). Values conductivity stomata, in all irrigation regimes, between the third and fourth measurements were significantly reduced. The measured values were: FI at 0,40 mol m-$^{-2}$ s^{-1} (H3) and 0,32 mol $m^{-2}s^{-1}$ (H4), RDI 0,38 mol $m^{-2}s^{-1}$ (H3) and 0.26 mol $m^{-2}s^{-1}$ (H4). The changes of dynamic conductivity of stomata was followed by canges in leaf water potential.Thus, the maximum values of water potential were the harvests H1 and then there was a decrease in the value of the harvest H4. Values water potential at the last harvest were: -1.01 MPa (FI) and -1.06 MPa (RDI).Results of the correlative relationships of parameters of water regime also indicate differences between the investigated season and so that are the chemical signals were more responsible for the reduction of conductivity stomatal cells in 2007. year, while in 2008. year decrease in conductivity stomata was accompanied by sheets of falling water potential (hydraulic signals drought).Reduced stomatal conductance in early stages of water stress inhibits transpiration rate more than it reduces the intercellular CO_2 concentration that is the driving factor for photosynthesis.Crop water use or transpiration (water diffusion to the air through the stomata) is mainly controlled by stomatal conductance. In terms of the efficient use of water in agriculture, stomatal response to the water status of the soil and plant system has been a critical area in crop physiology.

Reactions stomatal cells are of particular importance for reactions of plants to applied methods of irrigation. Such reactions stomatal cells is of particular importance because it allows the plant to dry when exposed to water more efficiently exploit the process of growth and metabolism and thus reduce the adverse effects of dehydration (Jones, 1980). According Maroco et al., (1997), stomata response to drought is more associated with the water content in the soil than with leaf water status. This indicates that the stomata respond to chemical signals which produces the root (Davies and Zhang, 1991). By reduction, in all irrigation

regimes, there is in the initial stages of the experiment, where a sudden drop in between the first and second measurement, the phase of flowering plants. This reduction in conductivity of the stomata was more pronounced than the reduction in water potential and this indicates that the chemical signals, rather than sheets of water regime (hydraulic signals) were more responsible for the reduction of conductivity stomata cells during this period The decline in conductivity stoma was reflected in the later stages of plant development 'and it is similar to the results that were obtained on potato Liu et al., (2005). These authors found that changes in the conductivity of the stoma by irrigation regimes applied in correlation with the interaction of chemical and hydraulic signals In the period in 2008. The decrease in conductivity of the stoma was accompanied by decreasing resources and leaves. Liu et al., (2004) in their results were given to the conductivity of the stoma in plants sensitive to desiccation of potato land in the initiation phase. Tuber and their growth, and these results confirm previous research on potatoes (Jefferies, 1989). Based on our results, we can assume that the degree of land drainage in the applied irrigation regime was sufficient to induce signals that are induced closure of stoma. It can also be assumed that the stoma closure was the result of induction of both types of signals, as well as their interactions.
However, recent investigations in field grapevines suggest if severe soil drying saving 50% water of control plants is applied during deficit irrigation feedback hydraulic signals
may play a dominant role (Santos et al., 2007; Rodrigues et al.,2008).

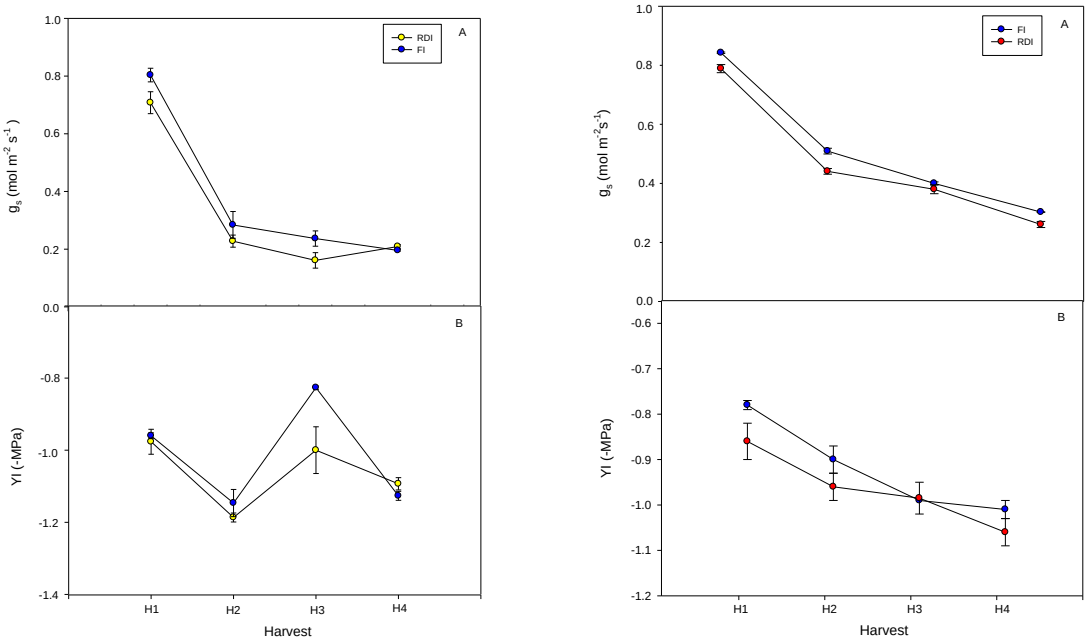
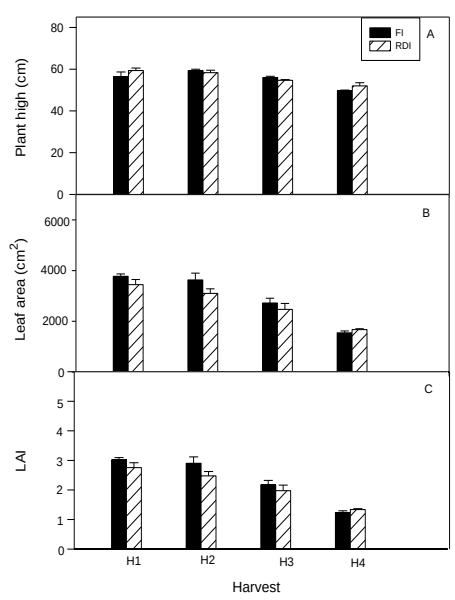
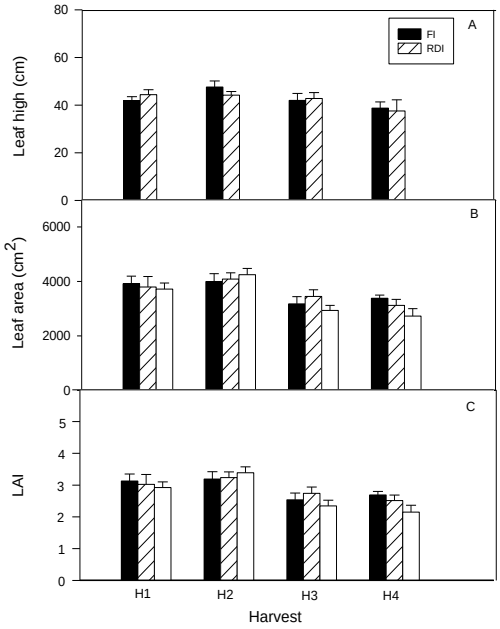


Fig 1. and 2. shows the changes in leaf water potential and stomatal conductivity of plants under the influence of FI and RDI irrigation regimes in 2007. and 2008. year

Plant height, leaf area and leaf area index (LAI)

Effects of irrigation regime (FI and RDI) on plant height , leaf area and leaf area index (LAI) during the 2007 are presented in figure 3.and 4. In our experiment there was no significant difference between FI and RDI irrigation regimes in plant height , leaf area and leaf area index during the 2007 year . Differences in values of these parameters have emerged within the regime, irrigation , between individual harvests . During the growing season expressed variation in leaf area between harvest H3 and H4 in all investigated regimes of irrigation during the 2007 years and this is probably due to the phenological phases in which the plants are contained, and this is the period of accumulation of dry weight in tubers .Plants were tested in both regimes of irrigation during 2007. year, were about the same height Maximum values of plant height were observed in all the studied irrigation regimes in the flowering stage in harvests H1 and H2. At the end of the experiment the height of FI and RDI plants was : 49.6cm and 52.0cm. The results showed that during the season in all investigated irrigation regimes were uniformly decline in plant heigh .The maximum value of the leaves area , in all investigated irrigation regimes were in the flowering stage , the harvest H1. FI and RDI irrigation regime values were : 3775 and 3446 cm^2 , and then there was a decline in the value to the end of the test period.This reduction was 40 % for FI plants, 32% for RDI plants . Leaf area, at the end of the experiment in irrigation regime FI and RDI were: 1544 cm2 , 1672 cm^2 . There was a reduction in leaf area at 8 % RDI irrigation regime in relation to optimally irrigated plants.Results for LAI values also show , for all irrigation regimes pronounced difference between harvest H3 and H4 and t-test was statistically highly significant difference ($P<0.001$) between individual harvests in FI and a significant difference ($P <0.01$) in the RDI of irrigation . LAI values for FI , RDI irrigation regime at the end of the experiment were : 1.23 and 1.33.In 2008. height of plants in FI and RDI was : 38.7 cm, 37.5 cm at the end of the experiment.Maximum values of the leaves area were at harvest H2 , at the stage of flowering potato plants in all investigated irrigation regimes . The FI values in the RDI were:3994, 4086cm^2, and then there was a decline in the value to the end of the test period . Leaf area in the RDI plants at the end of the experiment was : 3382cm^2, 3122 cm^2. Reduction of leaf area in RDI was 7.7 % compared to FI . Between 2008 the results show that the maximum values of these parameters for all irrigation regimes were in phonological fase of flowering plants and a decline in the value of going to the end of the growing season . RDI irrigation regime influenced the reduction in leaf area and LAI compared to FI plants . RDI irrigation regime has reduced the amount of irrigation water and leaf expansion thus avoid the effect of the excessive proliferation of plants .In the literature, there are a lot of controversial information to explain the effect on the LAI . Results Jamieson 's (1985) and Greenwood et al . , (1982) have shown that the application of irrigation deficit reduction in the LAI due to a decrease in potato transpiración surface and that this effect stimulates the development of roots and tubers. The measured maximum values for LAI , in all investigated irrigation regimes were at harvest H2 . In FI, RDI avalues were 3,11 ,and 3,20 , and then there was a gradual decline in values in all investigated irrigation regimes . The values of LAI at the end of the experiment in FI, RDI plants were : 2.69 and 2.51 . LAI in RDI plants was lower than the reduction in plant FI.RDi plants reduction was 6.7 % compared to the FI irrigation regime.





Fif.3 and 4. Effects of irrigation regime (FI and RDI) on plant height, leaf area and leaf area index during the 2007.and 2008.year

Conclusion

The results showed that that plants in regulated deficit irrigation at the end of each experimental season were exposed to increasing levels of stress. The decrease in plant water potential regime of regulated deficit irrigation also indicates that the water stress in the zone of the root system was sufficient to cause reduction of water potential of leaves. Measuring the impact of regulated deficit irrigation on stomata reaction showed that it was in 2007. there was a reduction of conductivity stomata in the regime of regulated deficit irrigation in the early stages of the experiment when there was still no change in water potential of leaves and this may indicate that chemical signals, (hydraulic signals) were more responsible reduction of conductivity stomatal cells during this period. In 2008.year decrease in conductivity stomata was accompanied by decrease in water potential of leaves of all investigated regimes of irrigation, which indicates a hydraulic signals drought. In the experiment in the field there were no significant differences between the effects of different irrigation regimes on plant height, leaf area and LAI at the end of the experiment, but the differences were demonstrated between individual harvests. Regulated deficit irrigation regimes influenced the reduction in leaf area and LAI in relation to optimally irrigated plants.

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BIOLOGICAL AND POMOLOGICAL CHARACTERISTICS OF CULTIVARS AND SELECTIONS OF APRICOTS FROM NOVI SAD (SERBIA) GROWN IN BANJA LUKA (BOSNIA AND HERZEGOVINA)

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Abstract

Growing apricot depends on many factors that lead to great variations in yields. The most common factors affecting the yield of apricots are extremely low winter temperatures, late spring frosts and the occurrence of apoplexy which causes drying out and decay of trees.

The aim of this paper is to compare biological and pomological characteristics of cultivars and selections from Novi Sad (Serbia) with apricot cultivars "Hungarian Best" (leading cultivar in the apricot orchards in the continental part of Bosnia and Herzegovina (BiH) in agro-ecological conditions of Banja Luka, BiH.

Tests were conducted during the period 2012-2014 at the plantation which was planted 2009 in Banja Luka. In the plantation there is following cultivars and selections of apricot: Aurora, NS-4, NS-6, Novosadska rodna, Novosadska kasnocvetna, SK-1, SK-3, SK-16a, SK-13a and Hungary best. Phenological stages of flowering and ripening was monitored. It was measured fruit weight, fruit length and width and stone weight. It was determined flesh/stone ratio and fruit shape index. Soluble solids contents were determined by refractometer.

All tested cultivars of apricots, besides Aurora, have achieved higher average fruit weight compared to the standard. Flesh/stone ratio of the fruit in a selection SK-13a and SK-16a was significantly higher than in Hungary Best cultivar. All studied cultivars ripened in the first half of July. The exception was Aurora cultivar, whose harvest was performed in mid-June.

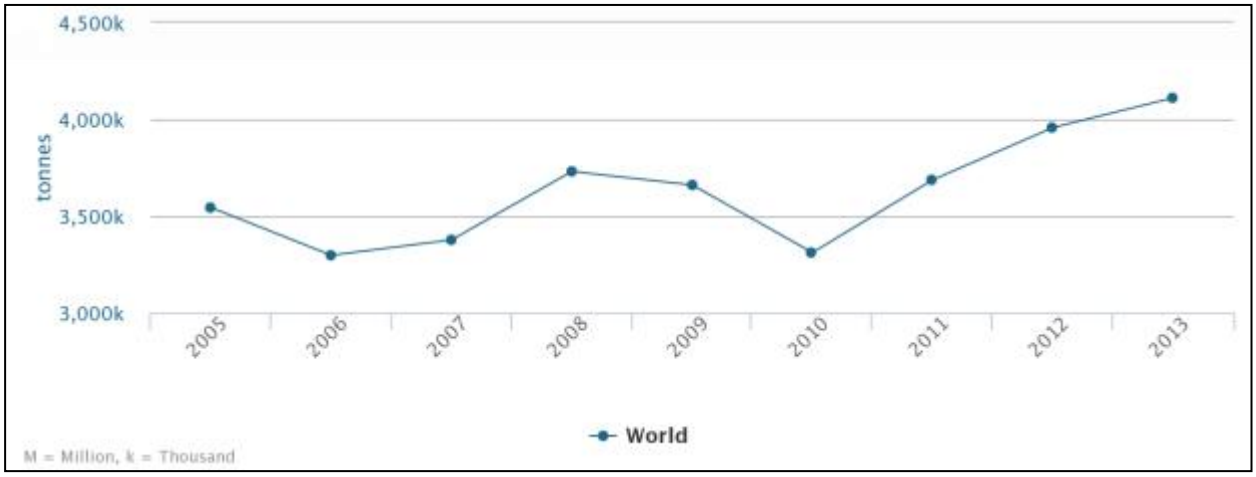
Of all tested cultivars and selections, only Aurora cultivar cannot be recommended for growing in continental conditions because it is very sensitive to low temperatures during the winter and often suffer from spring frosts. Other tested cultivars can be recommended for cultivation because being better in many indicators than standard cultivar.

Key words: *apricot, cultivar, flowering, fruit quality.*

Introduction

Apricot is a respectable fruit species that has a various usage and is particularly versatile use of the fruit. Fruits can be used for eating fresh or in the form of different products, such as jam, marmalade, compote, sweet, juice, dried fruits, apricot brandy and others. In varieties of apricot with a sweet taste kernel, the kernel according by its nutrient value and usefulness can be successfully used as a substitute for almonds, hazelnuts or walnuts in processing and confectionery industries.

The apricot production in the world is about 4.1 million tons (FAO, 2013). It is grown on about 504 000 ha (FAO, 2013). The largest producers are Turkey with 811,609 tons (FAO, 2013), which are 19.7% of total world production, followed by Iran and Uzbekistan. There is an evident tendency to increase production at the global level (Fig. 1). In Europe, the average annual production is 846 000 tons. The biggest producers are Italy, France, Spain and Greece.



Graph 1.The apricot production in the world in the period of 2005 - 2013

Situation in apricot production in our neighbouring countries is different. The average annual production in Serbia is 22952 tons with 1,8 million trees on approximately 3500 ha. The average yield of 13.9 kg per tree reflects mainly extensive growing of apricot (Djuric and Keserovic, 2007). In Croatia, according to Food and Agriculture Organization of the United Nationsfao (FAO) data, apricot is grown on about 400 hectares, with an annual production of about 1,000 tons.

Situation in apricot production in BiH is similar to the situation in Croatia. The average annual production is around 837 t. If we consider separately the Republic of Srpska (RS), according to data of the Statistical Office, apricot production is around 225t (2014), and in favourable years is around 400 t (2011). In plantations in 2014 there were 3400 trees and the average yield was 6.5 kg per tree. In favourable year yield is much higher, so in 2001 an average yield was 13.4 kg per tree.

The apricot production varies from year to year, as the most important causes of variations in production are: extremely low winter temperatures, occurrence of frosts in late spring and drying of trees (apoplexy).

To remedy the situation, in apricot production is necessary to implement a series of measures, primarily apricot need to be planted in appropriate agro-ecological conditions, use seedlings with intermediate, combined varieties in plantations, carry the summer pruning and irrigation (Keserovic et al., 2010).

There are around 2000 varieties of apricots, and in the last two decades has been created many new varieties (Milatovic, 2013). In the continental part of the RS and BiH in the apricot orchards and gardens, by representation, leading variety is Hungarian Best. In Serbia, more precisely, in Vojvodina province, where is favorable natural conditions for growing apricots, breeding process gave apricot variety and selections: NS-4, NS-6, NS rodna and NS kasnocvetna, SK-1, SK-3 , SK-5, SK-16a and SK-13a. These varieties are characterized by a later and longer blossoming, good yield, larger and better fruits than in Hungarian Best as the leading variety of apricot.

Knowing the state of apricot production in the RS and BiH, it is necessary to test biological, pomological and technological value of new varieties and selections of apricot in our agroecological conditions. That is the goal of this study. Based on results we can recommend which varieties should be given priority in growing new plantations of apricot.

Materials and Methods

The study of pomological characteristics of fruits of apricot varieties and selections were performed in 2013 and 2015 in the collection orchard of the Department for Fruit growing and Viticulture of Public Institution Agricultural Institute of Republic of Srpska, Banja Luka (Figure 1). Experimental orchard is planted in 2009. Cherry plum (*Prunus cerasifera* Ehrh.) was used as rootstock, and as intermediate plum cultivar Stanley was used. In the collection orchard there is 10 of trees of following apricot cultivars and selections: NS-4, NS-6, NS rodna and NS kasnocvetna, Aurora, SK-3, SK-16a, SK-13a, SK-1 and Hungarian Best as a standard variety. An average sample of 25 fruits of every variety has been taken in 2013 and 2015. We carried out following pomological measurements: fruit weight, width and height of the fruit and weight of kernel. It is also determined flesh ratio (%) and soluble solids content that is determined by refractometer. The exception is the variety Aurora, which didn't have fruits due to late spring frosts during the flowering in 2015.
The significance of differences was determined on the basis of an analysis of variance with the use of F test for two-factorial. For individual comparisons was used LSD test. Statistical analysis did not include the variety Aurora because it didn't have fruits in 2015.

Figure 1. Experimental orchard of apricot at Economy PI AIRS, Banja Luka (01/06/2015)

Results and Discussion

Table 1 shows average results of measurement of pomological-technological properties of fruits of apricot varieties and selections during the two-year test. In studies in Novi Sad (Rahovic et al. 2013) picking apricot varieties and selections was done earlier than in our research.
All the Novi Sad varieties and selections in 2013 had higher average fruit weight than the standard variety. The highest fruit weight in 2013 have made selections SK-13a (88.46 g) and SK-16a (88.47 g), while in 2015 the largest fruit weight achieved NS 4 (81,75g). In 2015, all studied varieties had higher fruit weight compared to the standard, except variety NS kasnocvetna and selection SK-16a. Statistical analysis of data for fruit weight in the two

observed years has shown statistically highly significant effect of the variety, the effect of year was not statistically significant, while the interaction effect of variety and year was statistically highly significant.

Tab1. Pomological and technological properties of the tested apricot variety and selections in 2013 and 2015, location of Banja Luka

VARIETY	Year	Date of harvest	Fruit weight (g)	Fruit width (cm)	Fruit height (cm)	Stone weight (g)	Flesh Ratio (%)	Soluble solids (%)
Aurora	2013	10.06.	45.6	3.92	3.74	2.50	94.52	17,25
	2015	-	-	-	-	-	-	-
NS 6	2013	08.07.	58,36	4.33	4.18	3.18	94.55	15.00
	2015	10.07.	73,71	5.30	5.17	2.66	96.39	19:50
SK-3	2013	11.07.	73.74	4.92	4.50	2.40	96.74	15.00
	2015	10.07.	77.73	5.42	5.04	2.39	96.93	17,66
SK-13a	2013	11.07.	88.46	5.49	5.27	2.58	97.08	15,25
	2015	10.07.	75,50	5.22	5.17	2.14	97.16	20.66
NS Kasnocvetna	2013	11.07.	62.98	4.96	4.95	4.20	93.33	15.00
	2015	10.07.	52.72	4.74	4.72	3.59	93.19	22.66
SK-16a	2013	11.07.	88.47	5.54	5.35	2.55	97.11	17,00
	2015	10.07.	57.86	4.77	4.90	2.25	96.11	19,16
NS 4	2013	12.07.	67.78	5.15	4.96	2.15	96.83	15,50
	2015	15.07.	81.75	5.46	5.48	3.20	96.08	19,33
NS rodna	2013	12.07.	81.46	5.38	5.34	2.93	96,40	15,50
	2015	15.07.	74.94	5.28	5.26	3.31	95.58	20,33
SK-1	2013	12.07.	82.81	5.48	5.32	2.78	96.64	15,20
	2015	10.07.	74.81	5.28	5.20	3.10	95,85	20.66
Hungarian Best	2013	15.07.	51.26	4.66	4.60	2.18	95.75	16,00
	2015	13.07.	66,44	5.18	4.98	3.15	95.25	17,66

NS 6 in 2013 and NS kasnocvetna and SK-16a in 2015 had lower values for height and width of the fruit compared to the standard. The highest flesh ratio in 2013 had a selection of SK 16a (97.11%), and SK - 13a (97.16%) in 2015.

All cultivars in 2015 have achieved higher soluble solids compared to 2013. The highest content of soluble solids in 2015 had NS Kasnocvetna (22.66%), while the lowest content of soluble solids had cultivar SK -3 (17.66%) and the standard variety.

Results of statistical data analysis for pomological and technological properties of the fruit in a two-year research are given in Table 2.

The highest average fruit weight in the two-year research has made selections SK - 13a (81.98 g), while the lowest fruit weight had variety NS kasnocvetna (57.85 g). The highest coefficient of variation for fruit weight had variety Hungarian Best (30%). Results of weight in varieties NS 6 NS 4 and NS rodna were similar to the results of research in the region of Belgrade (Milatovic *et a.* 2015) All varieties except NS kasnocvetna had higher fruit weight than Hungarian Best, which is the standard variety. The highest average fruit width in the years of research had selections SK-1 (5.38 cm) which also had the second highest fruit height (5.26 cm). Minimum width and height of the fruit had NS 6 (4.82 cm and 4.68 cm). All tested varieties, except the varieties NS 6 and NS kasnocvetna, had a greater width of the fruit compared to the standard variety (Hungarian Best). Most varieties has reached a greater height of the fruit compared to the standard, except for varieties NS 6 and SK-3. NS rodna and NS 4 have achieved similar values for the width and height of the fruit as in research in the area of Belgrade (Milatović *et al.* in 2015), while the cultivar NS - 6 achieved a lower value. In conperation to our study, Mratinić *et al.* (2010) recorded similar measurements of height and width of fruit in NS 4 variety. Statistical analysis of the data for width of the fruit in two years there was a statistically highly significant effect of variety, the effect of year is

statistically significant, while the interaction effect between variety and year statistically highly significant.

Tab2. Pomological and technological properties of the tested apricot variety and selections in, location of Banja Luka (average 2013 and 2015)

VARIETY	Fruit Weight (g)	CV (%)	Fruit width (cm)	CV (%)	Fruit height (cm)	CV (%)	Stone weight (g)	Flesh Ratio (%)	Soluble solids (%)
NS 6	66,04	16.1	4.82	12.8	4.68	12.2	2.92	95.47	17,25
SK-3	75.74	19.1	5.17	7.8	4.77	8.6	2.40	96.84	16,33
SK-13a	**81.98**	18.3	5.36	8.3	5.22	7.7	2.36	**97.12**	17,96
NS Kasnocvetna	57,85	16	4.85	5.1	4.84	5.7	**3.90**	93.26	**18,83**
SK-16a	73.17	26.9	5.16	10.2	5.13	8.2	2.40	96.61	18,08
NS 4	74.77	14.9	5.31	6	5.22	7.6	2.68	96.46	17,42
NS rodna	78,20	14	5.33	6.8	**5.30**	5.8	3.12	95,99	17,92
SK-1	78,81	13.8	**5.38**	6.1	5.26	5.9	2.94	96.25	17,93
Hungarian Best	58.85	30	4.92	10.3	4.79	8.5	2.67	95,50	16.83
Average varieties	69,10		5.02		4.89		2.79	95.71	17,58
Average year									
2013	72,62		5.10		4.93		2.77	96.05	15,49
2015	70.61		5.19		5.10		2.87	95.84	19,74
LSD (years)									
0.05	50,81		1.35		1.21				
0.01	66.78		1.78		1.59				
LSD (variety)									
0.05	107.79		2.87		2.57				
0.01	141.65		3.77		3.37				

The largest coefficient of variation for the fruit width had NS 6 (12.8%) and the smallest NS kasnocvetna (5.1%). For the height of fruit the largest coefficient of variation had a NS 6 (12.2%) and the smallest NS kasnocvetna (5.7%). Statistical analysis of data for the height of the fruit in two years showed that there was a statistically highly significant effect of variety, effect of year and the effect of the interaction between variety and the year.

The highest kernel weight had the NS Kasnocvetna (3.90 g), and the smallest selection SK-13a (2.36 g). The average weight of kernel of all tested varieties was 2.79 g. The average flesh ratio of all tested varieties was 95.71%. The highest flesh ratio had a selection of SK 3 (97.12%), wich was higher compared to research in Vojvodina (Rahovic *et al.,* 2013). The lowest flesh ratio was recorded in the variety NS Kasnocvetna (93.26%). Most varieties in the experiment achieved a higher flesh ratio compared to the standard. Exceptions are varieties NS Kasnocvetna and NS 6.

The highest average percentage of soluble solids had NS Kasnocvetna (18.83%) and the smallest selection SK - 3 (16,33%), which is also the only one that had a lower soluble solids content than standard varieties. In our research, the variety NS 4, NS 6 and NS rodna have achieved a higher content of soluble solids in relation to research in the area of Belgrade (Mratinic *et al.,* 2010; Milatovic *et al.,* 2015).

Conclusion

Based on two years study (2013 and 2015) pomological and technological characteristics of variety and selections of apricot from Novi Sad in agroecological conditions of Banja Luka can be concluded:

- The ripening of the fruit is average was in the second decade of July;
- Most of the tested varieties achieved an average higher fruit weight compared to the standard cultivar Hungarian Best. Varieties SK-13, SK-1 and NS rodna stand out by size of fruits.
- Average values of fruit dimensions (width and height) are generally higher than the standard variety. The exception is the NS 6, which has a lower value;
- Largest kernel has NS Kasnocvetna which contributed that varieties achieve the most unfavorable flesh ratio (93.26%);
- Tested apricot cultivars and selections are generally performed better flesh ratio compared to the standard variety. In particular selection SK-13a (97.12%), SK-3 (96.84%) and SK-16a (96.61%) stand out;
- The content of soluble solids in 2015 was higher compared to 2013. Flesh ratio in two-year test ranged from 16.83% (standard variety) to 18.83% (NS Kasnocvetna). All study apricot cultivars and selections had higher soluble solids compared to the standard.
- Varieties NS rodna, NS 4 and selection SK-16, SK-13a and SK-1 stand out in results of fruit quality;
- Variety Aurora due to very early flowering and sensitivity to low temperatures during the winter is not suitable for cultivation in an area with a continental climate.

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USE OF SPENT MUSHROOM SUBSTRATE FOR GROWING GERANIUM (Pelargonium peltatum L.) AND SURFINIA (Petunia hybrida Juss.) SEEDLINGS

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Abstract

After mushrooms are harvested a large amount of used compost that has a high content of organic matter and the rich mineral composition remains. Such accumulated compost is a good material to improve the physical properties of soils and plant nutrition. The aim of this study was to investigate the possibility and feasibility of using spent mushroom compost from *Agaricus bisporus* as an alternative growing media for greenhouse production of *Pelargonium peltatum* L. and *Petunia hybrida* Juss. seedlings. The experiment was conducted under greenhouse conditions at the Faculty of Agriculture, University of Banja Luka (Republic of Srpska - RS, Bosnia and Herzegovina - BiH) in 2014. It was arranged in a split - plot design with four replicates and two treatments (commercial substrate and mixture of commercial substrate and spent mushroom compost) for each variety. Morphological parameters, were recorded regularly during growth and development of seedlings. At the end of experiment, seedlings were sampled and root and above-ground fresh and dry weight were recorded. Obtained results showed significantly higher values of all investigated parameters of seedlings grown on spent mushroom compost. Thus it can be concluded that use of spent mushroom compost, as additional of commercial substrate in the production of geranium and surfinia seedlings is possible and economically justified as well as environmental friendly.

Key words: alternative substrate, geranium, surfinia, seedlings.

Introduction

Peat is the most widely used substrate for the cultivation of horticultural plants, but its high price and increasing exploitation, lead producers for the search of alternative substrate or at least their partial replacement. As one of the alternatives in the production of flowers and vegetables, it can be used compost from microbial decomposition of various organic substances (Karasek, 2002). Mushroom production is becoming more popular in our environment. The most important advantage of this branch of agriculture is that it can all take advantage of the production cycle. After mushrooms are harvested a large amount of used compost that has a high content of organic matter and the rich mineral composition remains (Lazic et al., 2001). Such accumulated compost is a good material to improve the physical properties of soils and plant nutrition. Numerous researchers confirms that disused mushroom compost can be used as substrate for successful cultivation of many crops (Maher, 1994; Chong et al., 1994; Polat et al., 2009; Gonani et al., 2011). However, care should be taken on the amount of used mushroom compost. Lemaire et al. (1985) reported that the used mushroom compost can't be used alone due to weak water permeability, high salinity and a neutral pH which is not suitable for all horticultural plants. Çelikel and Çağlar (1997) reported that higher yield and earliness for tomato and cucumber growth were found in mixtures of peat and spent mushroom compost (1:1) than the plants that were grown in a garden soil.

Therefore, the aim of this study was to determine the effectiveness of the use of spent mushroom compost from growing mushrooms - *Agaricus bisporus* in the production of geranium (*Pelargonium peltatum* L.) and surfinia (*Petunia hybrida* Juss.) seedlings.

Material and methods

The study was conducted in greenhouses on the family farm Šušak in Prnjavor municipality, and in greenhouses of Faculty of Agriculture, University of Banja Luka (RS/BiH) in 2014 period. As an initial planting materials were used already rooted cuttings of geranium (*Pelargonium peltatum* L.) and surfinia (*Petunia hybrida* Juss.). For rooting cuttings were used a commercial substrate suitable for sowing seeds and cuttings Fruhstorfer Erde type: *Aussaat und Stecklingserde* from Hawita EU manufacturer. The substrate contains perlite for better aeration and friability. Other substrate characteristics are: pH 5.9; N mg/l=80; P_2O_5 mg/l=60; K_2O mg/l=90; EC 50 ms l; retention capacity 700 ml/l. Experiment was set up as a spli-plot design with four replications and two treatments - a commercial substrate (A1) and a mixture of spent mushroom compost and commercial substrate at a ratio of 70:30 (A2). The trial consisted of a total of 80 plants, for each variety, which are divided into two groups with 40 plants in each group and 10 plants per replication. Commercial substrate: Klasmann-Deilmann substrate - *TS 3* which is suitable for pricking out seedlings, pH 5.5-6.5 and that is enriched with NPK fertilizer 14:16:18 in the amount of 1.0-1.5 kg/m^3 was used as a control for geranium seedlings. Commercial substrate: *Baltisches substrat* from Hawita EU manufacturer, enriched with Fe as such is particularly suitable for plants like surfinia and other from familly *Solanaceae*, with reduced pH 5.2 was used as a control for surfinia seedlings. Spent mushroom compost was previously mixed with garden soil and composted for six months and was used as a treatment for both variety. In the laboratory of Soil Science, Faculty of Agriculture, University of Banja Luka chemical analysis of composted substrate was conducted and its composition is given in Table 1.

Table 1. Agrochemical analysis of spent mushroom compost

pH		organic matter %	AL-P_2O_5 mg/100g	AL-K_2O mg/100g
H_2O	KCl			
7,32	7,00	14,3	72,0	280

During the experiment was carried out measurements of morphological indicators of growth and development of plants (plant height, number of leaves, number of flowers and number of flower branches). In order to examine the growth and development of roots and above-ground parts of seedlings under the affect of different substrates, the root was cleaned from the substrate, washed with distilled water, dried with paper towels, then was measured fresh weight of above-ground parts and roots of each plant separately. Weighed above-ground parts and roots are packed in separate paper bags, properly labeled and placed in an oven to dry. Drying the plant material is lasted to a constant weight at a temperature of 70^0C, after which it was carried out the weighing of dry roots and above ground parts. The mass of fresh and dry matter was measured and expressed in grams (g). The obtained data were statistically analyzed using analysis of variance and the differences between specific substrate used F - test, using a computer program VVSTAT (Vukadinovic, 1994).

Result and discussion

In order to determine the influence of the alternative substrate on the growth and development of geranium (*Pelargonium peltatum* L.) and surfinia (*Petunia hybrida* Juss.) seedlings through plants morphological indicators and the fresh and dry weight of roots and above-ground parts, the following results were obtained. During the experiment, measurements were made of morphological indicators of plant growth and development. Table 2. and Table 3. shows the average value of the indicator. Plants treatment showed better results in terms of morphological characteristics (plant height, leaf number, number of flowers, number of

flowering branches), as the average values of all investigated parameters were significantly higher than the average value of the control plants.

Table 2. Influence of spent mushroom compost on morphological indicators of growth and development of geranium seedlings (Pelargonium peltatum L.*)*

Treatment variant (A)	*Plant height*	*Number of leaves*	*Number of flowers*	*Number of flower branchs*
Control (A1)	7,60[a]	9,74[a]	0,89[a]	1,01[a]
Treatment (A2)	9,61[b]	12,02[b]	1,45[b]	1,21[b]
Average	8,60	10,88	1,17	1,11
Analysis of variance - F	*32,31***	*26,29***	*31,91***	*12,49**
LSD	*Plant height*	*Number of leaves*	*Number of flowers*	*Number of flower branchs*
0,05	*0,9816*	*1,2343*	*0,2768*	*0,1597*
0,01	*1,6280*	*2,0471*	*0,4591*	*ns*

Plant height, number of leaves and number of flowers of geranium seadlings were under a very significant ($p \leq 0.01$) influence of the substrate. Highest recorded plant height 9.61 cm belong to variant A2, while lowest 7.60 cm belonging to the control plants (A1). The highest average number of leaves (12.02) was determined in A2 variant, and the lowest of 9.74 belonging to the control plants, whereby the difference between the variant was 23% in favor of variant A2. The highest average number of flowers of the treatment was 1.45, which was 63% more flowers than in the control group of plants (A1 0.89). Number of flower branches was under significant ($p \leq 0.05$) influence of the substrate. The highest average number of flowerbranches of the treatment was 1.21, which was 20% more flower branches than in the control group of plants (1.01) (Tabela 2.).

Table 3. Influence of spent mushroom compost on morphological indicators of growth and development of surfinia seedlings (Petunia hybrida Juss.)

Treatment variant (A)	*Plant height*	*Number of leaves*	*Number of flowers*	*Number of flower branchs*
Control (A1)	16,06[a]	25,97[a]	0,64[a]	7,25[a]
Treatment (A2)	17,20[b]	28,05[b]	1,16[b]	8,79[b]
Average	16,63	27,01	0,90	8,02
Analysis of variance - F	*11,98**	*22,75***	*25,28***	*16,02***
LSD	*Plant height*	*Number of leaves*	*Number of flowers*	*Number of flower branchs*
0,05	*0,8131*	*1,0671*	*0,2531*	*0,9384*
0,01	*ns*	*1,6166*	*0,3834*	*1,4217*

Plant hight was under significant ($p \leq 0.05$) influence of the substrate. The highest average value of the plant height was 17.20 cm and was observed in plants treatment, and the difference between the control group of plants and the treatment was 7% in favor of the treatment group. Treatment with the substrate had a significant ($p \leq 0.01$) impact on the number of leaves, number of flowers and number of flower branches of surfinia seedlings. The highest

average number of leaves (28.05) was determined in A2 variant, and the lowest of 25.97 belonging to the control plants, whereby the difference between the variant was 8% in favor of variant A2. The highest average number of flowers of the treatment was 1.16, which was 81% more flowers than in the control group of plants. The highest average number of flower branches was determined in A2 variant, and the lowest of 7.25 belonging to the control plants, whereby the difference between the variant was 21% in favor of variant A2 (Table 3.).
After analyzing the morphological indicators of growth and development of geranium (*Pelargonium peltatum* L.) an surfinia (*Petunia hybrida* Juss.) seadlings, measurement of fresh and dry weight of plants were carried out and the obtained results are shown in Table 4. and Table 5.

Table 4. Influence of spent mushroom compost on fresh and dry above-ground part and root mass of geranium seedlings (Pelargonium peltatum L.)

Treatment variant (A)	*Fresh above-ground mass (g)*	*Dry above-ground mass (g)*	*Fresh root mass (g)*	*Dry root mass (g)*
Control (A1)	18,00[a]	2,31[a]	2,25[a]	0,40[a]
Treatment (A2)	23,67[b]	2,83[b]	2,50[b]	0,43[a]
Average	20,83	2,56	2,37	0,42
Analysis of variance - F	*25,55***	*8,28**	*37,49***	*0,19*
LSD	*Fresh above-ground mass (g)*	*Dry above-ground mass (g)*	*Fresh root mass (g)*	*Dry root mass (g)*
0,05	*3,1123*	*0,4951*	*0,1133*	*ns*
0,01	*5,1617*	*ns*	*0,1880*	*ns*

Based on the data of Table 3, it can be seen that the fresh mass of above-ground part was under a very significant ($p \leq 0.01$) influence of the substrate. The highest recorded average value 23.67 g belonged to the treatment (A2). The lowest recorded value was 18.00 g and belonged to the A1 variant. Dry above-ground parts mass, was under significant ($p \leq 0.05$) influence of the substrate. The greatest value of the dry weight of above-ground parts belonged to the variant A2 (treatment with the substrate) 2.83 g in relation to variant A1 (control) 2.31 g. Fresh root mass, was also under very significant ($p \leq 0,01$) influence of the substrate. Highest recorded value was in A2 variant plants 2.50 g compared to lowest recorded value in variant A1 (2.25 g). Statistical significance was missed in dry mass of roots (Table 4).

Table 5. Influence of spent mushroom compost on fresh and dry above-ground part and root mass of geranium seedlings (Petunia hybrida Juss.)

Treatment variant (A)	*Fresh above-ground mass (g)*	*Dry above-ground mass (g)*	*Fresh root mass (g)*	*Dry root mass (g)*
Control (A1)	18,50[a]	1,88[a]	1,41[a]	0,21[a]
Treatment (A2)	19,32[b]	2,11[b]	3,53[b]	0,33[b]
Average	18,91	2,00	2,47	0,27
Analysis of variance - F	*6,00**	*12,31**	*34,34***	*10,42*[*]

LSD	*Fresh above-ground mass (g)*	*Dry above-ground mass (g)*	*Fresh root mass (g)*	*Dry root mass (g)*
0,05	*0,8216*	*0,1587*	*0,8874*	*0,0928*
0,01	*ns*	*ns*	*1,3443*	*ns*

The average fresh and dry weight of above ground part were under the statistically significant ($p \leq 0.05$) influence of the substrate. The highest recorded average fresh weight value of treatment was 19.32 g and dry weight value was 2.11 g. The lowest recorded value were 18.50 g and 1.88 g and belonged to the A1 variant from both variant. Fresh root mass was under very significant ($p \leq 0.01$) influence of the substrate. Highest recorded value was in A2 variant plants 3.53 g compared to lowest recorded value in variant A1 (1.41 g). Dry root mass was under significant ($p \leq 0.05$) influence of the substrate. The highest value of the dry weight of root belonged to the variant A2 (treatment with the substrate) 0.33 g in relation to variant A1 (control) 0.21 g. (Table 5).

Pine bark and peat formed by decomposition of *Sphagnum* moss are the two most commonly used substrate components that are currently used for the production of horticultural plants. Although many times so far it’s proven the effectiveness and justification of the use of these substrates, the developed countries are increasingly using alternative natural substrates. This is due to high cost and availability of existing substrates, as well as unplanned use of forests and environmental degradation (Wright et al., 2009). That is why the alternative substrates are very important in a container production of horticultural crops. Any potential replacement for peat must have the appropriate physical and chemical characteristics, must be available in significant amounts, consistent and compatible with the economic potential markets, and one of the materials that meet these requirements are coconut fibers (Evans and Stamps, 1996). The use of agricultural waste and other composted materials in replacement for peat and pine bark is not new in the world, but among local producers this is a novelty. Also, the use of organic fertilizer, has great application in improving the content of nutrients in the soil. After harvesting the mushrooms, millions of tons of "spent" substrates become available for other purposes. The use of this substrate has a number of advantages, which are recognized by many plant producers, but they also depend on the types of mushrooms that are grown. Delgado et al. (2008) compared the cultivation of geranium in commercial compost with peat and the substrate which was enriched with 10% of chicken feces, where it came to the result that after three months of growing plants on a substrate with chicken feces increased number of leaves, number of flowers and stem length, and thus increased dry weight of the overhead part of geraniumwere recorded. In the cultivation of marigold (*Tagetes patula* L.) does not recommend the use of spent mushroom compost in an amount greater than 50% relative to the amount of commercial substrates, due to the impossibility of controlling the substrate and the low salinity water capacity (Young et al. 2002). Çiçek et al. (2014) studied the impact of fresh and spent substrate from mushroom growing on the growth of chrysanthemum (*Chrysanthemum morifolium* 'Vista'). The best results have indicated a mixture containing 12.5% of fresh substrate and 25% of spent substrate of mushroom growing.

Conclusion

This study confirmed that the spent mushroom compost can be used in the production of *Pelargonium peltatum* L. and *Petunia hybrida* Juss. seedlings as a supplement to commercial compost in the amount of 70%. Efficient use of spent mushroom compost is reflected in an increase in the average measured value of morphological indicators of growth and development (plant height, leaf number, number of flowers and number of flowering branches) and the increase in fresh dry mass of above ground parts or root relative to the average value of control plants seedlings. Based on the results, it can be concluded that the

use of spent mushroom compost from growing mushrooms (button mushrooms - *Agaricus bisporus*), as well as the addition of a certain amount of commercial substrates in the preparation of alternative substrates in the production *Pelargonium peltatum* L. and *Petunia hybrida* Juss. seedlings is recommended because of a positive impact on the growth and development of roots and above-ground parts, and with all that is economically viable and suitable for the environment.

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APPLICATION EFFECT OF MICROBIOLOGICAL FERTILIZER ON PHYSICAL AND CHEMICAL PROPERTIES OF FRENCH BEAN (Phaseolus vulgaris L. ssp. vulgaris)

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Abstract

Modern agricultural production implies irrational application of large amounts of pesticides and fertilizers, which significantly reduces the quality and fertility of agricultural land, adversely affect the environment and reflect on food quality and human health. French beans (*Phaseolus vulgaris L.*) is the crop which in our production conditions are grown in the greenhouse as early in the spring or autumn that matures in October. The aim of this study was to investigate the effect of microbiological fertilizer individually and in combination with mineral fertilizer on the physical and chemical properties of the fruit French beans. Sampling as well as fruit length, width and weight were recorded at the stage of full maturity. As part of chemical analysis of the fruit, total content of nitrogen, nitrate, protein and dry matter was determined. According to the results of research the largest value of analyzed physical parameter were registered in variety of microbiological fertilizer in combination with mineral one. The largest values of chemical composition parameter of French beans fruits were registered in variety with only mineral fertilizer. Significantly, the lowest nitrate content was observed in variants with an example of only microbiological fertilizer applied.

Key words: *French bean, greenhouse, fertilizer.*

Introduction

French beans (*Phaseolus vulgaris L.*) belong to the family *Fabaceae*. Peas and beans are most spread species from the genus *Phaseolus* and occupying more than 85% of the areas under all the species of this genus in the world and can be regarded as varieties of the same plant species *Phaseolus vulgaris L.* (*Satiriou and Traka-Mavrona, 2008; Baćanović, 2010*).

French beans (*Phaseolus vulgaris*) is the crop which are grown in the greenhouse as early in the spring or autumn that matures in October (*Đurovka, 2006*). It is grown because of pods that represent useful part of human diet because of their low energy value and wealth of mineral substances (*Zdravković, 2000*). Legumes, including beans are the primary source of proteins in the human diet. Also, it features a wealth of carbohydrate and minerals (*Lott et al., 2000*), and is a good source of Ca, Fe and vitamin C (*Grubben, 1977; Almeida Costa et al., 2006*). French beans can be grown in all soil types, from sandy loam to clay soil (*Indrakumar Singh, 2009*). At the root of beans and other legumes, are bacteria of the genus *Rhizobium* which fix atmospheric nitrogen in the process of nitrogen fixation rendering it into ammonia form that is accessible to plants and microorganisms. Microbiological preparations used as a microbiological fertilizer are containing selected cultures of microorganisms that are used to inoculate the seeds and seedlings or are introduced in the soil in order to intensify certain microbiological processes that increase the content of available nutrients, and in leguminous plants can be used as the sole feed (*Djurić, 2008*). The increasing cultivation of plants without using the fertilizers and pesticides, as well as the ability of microorganisms to provide the necessary plant nutrient, indicating that the microbiological fertilizer occupy an important place in agricultural production. (*Tyler et al., 2008*). French beans responds well to inoculation of the seed with bacterial fertilizers (*Jarak et al., 2010*). Inoculation of legume

seed is well studied, and the exploitation of these beneficial symbiotic communities is a mark of successfully applied agricultural microbiology (*Alves et al., 2003*). Microbiological preparations when used in vegetable production, as individual or combined cultures, have positive impact on the length of the plant (stem and roots), plant weight (raw and dry), nitrogen content in the plant, quality and yield (*Djurić, 2008*).
Modern agricultural production means irrational application of large amounts of pesticides and fertilizers, which significantly affects the quality and fertility of agricultural land, adversely affect the environment, which affects the quality of the food and the health of people (*Marinković et al., 2014*). Therefore, the aim of the study was to investigate the effect of microbiological fertilizer individually and in combination with mineral fertilizer on the yield and chemical composition of French beans.

Materials and methods

The survey was conducted in 2014 in the greenhouse of 360 m^2, in the village of Hodbina, Mostar (Bosnia and Herzegovina). The experiment used the seed of domestic yellow beans that were planted in the seeding substrate Potgraund H 20.08.2014. Just before sowing the seed part is immersed in microbiological fertilizer, a combination of six bacteria belonging to the nitrogen-fixing-*Azotobacter chroococcum A. vinelandii, Derxia sp*. and mineralizers phosphorus of *Bacillus licheniformis, B. subtilis, B. megaterium*.
Planting seedlings is done 26.08.2014. Plants are planted in double strips, where in the distance between the strips was 80 cm and 50 cm spacing in the row and 40 cm between rows. The trial was a randomized complete block design with 4 replications. Variations on the basic experimental plots are as follows: 1. Microbiological fertilizer 2. Microbiological fertilizer+mineral, 3. Mineral fertilizer and 4.Control.
The method of cultivation in these variants differed mainly in the diet. Alternative 1 was adopted microbial fertilizer which is applied to the root. Another variant is contained microbial fertilizer addition crystalline formulation of fertilizer 20:20:20, 15:30:15, 15:5:30 pm. The third variant was used as fertilizers. The control variant was not fertilized. Protection of French beans implied usual methodology for greenhouse production of beans in this area. At the stage of full maturity sampling was done (14.10. 2014) and weight, width and length of 40 pods per variant was determined. As part of the chemical analysis of the fruit is determined by the content of total nitrogen (the Kjeldhal), nitrate (spectrophotometry), the content of dry matter (drying oven to constant weight to 130^0C) and proteins (from nitrogen). The results were processed with statistical method of analysis of variance and tested with LSD test.



Picture 1: Planting of beans Picture 2: Flowering phase Picture 3: Measuring the length

Results and discussion

According to the survey (Table 1.), It can be seen that the values of the physical parameters of the largest varieties of beans Microbiological + Mineral, a minimum width in variants only microbiological fertilizer, while the remaining two physical parameters (weight, length) was the lowest in the control treatment. Results of analysis of variance with the physical parameters of beans showed that the investigational factor had statistically significant effect on the weight and length of pods, while the width of the pods that effect was not statistically significant. According to Tukey test weight and length husk was statistically significantly higher in variants Microbiological+Mineral concerning variant only microbiologically and control. Between the varieties of microbiological control and there was no statistical significance. Also, while the difference in weight and length of the follicle was not statistically significant between the Microbiological+Mineral and Mineral.

Table 1. The effect of the fertilization on the physical parameters of French beans

Variety	Weight (g)	Length (cm)	Width (cm)
Microbiological	11,39[b]	18,66[b]	1,39
Microbio.+ Mineral	14,65[a]	20,64[a]	1,6
Mineral	13,87[a]	19,81[a]	1,54
Control	10,86[b]	18,51[b]	1,42
$W_{0,05}$	1,18	0,84	ns
$W_{0,01}$	1,56	1,11	ns

Table 2. The effect of the fertilization on the chemical parameters French beans

Variety	Nitrogen (%)	Nitrate (mg/kg)	Proteins (%)	Dry matter content (%)
Microbiological	0,16[b]	242,54[b] (*)	0,99[b]	7,95
Microbio.+ Mineral	0,19[b]	539,1[a]	1,2[b]	8,05
Mineral	0,59[a]	540,19[a]	3,71[a]	8,4
Control	0,14[b]	285,21[b]	0,87[b]	8,1
$W_{0,05}$	0,19	204,45	1,45	ns
$W_{0,01}$	0,29	309,63	2,20	ns

According to the results of analysis (Table 2.) of variance test factor had also an impact on the chemical parameters beans, statistically significant effect on the content of peat, significantly the protein content and nitrate, while the dry matter content was no statistical significance of the test parameters.

Previous studies the application of inoculation in the production of green beans has shown that inoculation of bean seeds individual strains of rhizobium, or mixtures thereof, provide nitrogen almost completely, while nodulation, number of legume, nitrogen content in the grain and the yield depends of the different sort of beans and characteristics of rhizobium (*Marinković, 2006*).

Highest content of nitrogen and protein was observed in variants with mineral fertilizer, which is, according to the Tukey test, statistically highly significant in comparison to the other two variants, between which there was no statistical significance. According to research *Almeida Costa et al, 2006*, in French beans protein content ranged from 1.7 to 1.9 g / 100 g. The smallest content of nitrate is observed in only variant microbial fertilizer, then in the

control according to the Tukey test statistically significantly less than in the other three variants, between which there was no statistical significance of the values of the test parameters.

Conclusion

The results showed that microbiological fertilizer in combination with mineral has positive impact on the physical parameters of beans. Weight and length of pods have the highest value in the variant microbiological + mineral fertilizer, while value in the control variant was the lowest. Width value of French beans was uniform in all variants and it is not statistically significant.

All tested chemical parameters have the highest values in the variant of mineral fertilization.

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TECHNOLOGICAL CHARACTERISTICS OF BLACK WINE VARIETIES IN THE CONDITIONS OF THE TREBINJE (BOSNIA AND HERZEGOVINA) VINEYARD

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Abstract

The aim of this paper is to present technological characteristics of four black wine varieties (Pinot Noir, Merlot, Vranac and Cabernet Sauvignon) in the conditions of the Trebinje vineyard during the vegetation in 2013. Research of technological characteristics include mechanical compounds of grapes and berries as well as quality of the grapes and berries (sugar, acid presence and pH). The biggest mass of the cluster, grape peduncle and berries in a cluster as well as the biggest mass of 100 berries and mass of 100 berries flesh had Vranac variety while the smallest had Cabernet Sauvignon variety. The biggest mass of berries skin and mass of seeds in 100 berries had Vranac variety and the smallest had Pinot Noir variety. Sugar content varied between 17.9% (Vranac) and 23.7% (Merlot). Vranac variety had the smallest acid content (3.6 g l^{-1}) while Cabernet Sauvignon variety had the biggest (5.9 g l^{-1}). The smallest pH also had Vranac variety (3.08) while the biggest had Merlot (3.37).

Keywords: *technological characteristics, mechanical compounds of grapes and berries, grapes quality, Trebinje vineyards*

Introduction

Selection of appropriate grape variety has a great importance in modern viticulture (*Kerridge et Antiliff*, 2004). In the area of Herzegovina there is large a number of varieties, out of which some are native (Žilavaka, Blatina, Trnjak, Bena etc.), while a certain number of varieties is introduced. Among introduced varieties the most important are Smederevka, Prokupec, Vranac and Plavdina. In the last decade the renovation of vineyards in Herzegovina started so some other varieties such as Merlot, Cabernet Sauvignon, Pinot Noir, Muscat, Shiraz, Chardonnay and others are introduced. The aim of this paper is to study the economic and technological characteristics of four black wine varieties (Pinot Noir, Merlot, Vranac and Cabernet Sauvignon) that were grafted on vineyard Berlandieri × Riparia Kober 5BB in agroecological conditions of Trebinje.

Material and methods

The study of economic and technological characteristics of the studied grapevine varieties is done during the vegetation of 2013. The experiment was set up at the location of Zasad polje, Trebinje. The vineyard was established in 2008 at an altitude of 273 m. Planting distance was 2.4 x 0.9 m. During the research year in experimental orchards, basic measures of pruning were applied, as well as protection from the most important causes of diseases and pests. During the experiment, the orchard was irrigated by the "drop by drop" method.

As for the economic and technological characteristics of tested varieties, mechanical composition of cluster and berries and chemical composition of stum was determined. The mechanical analysis of cluster and berries determined the following indicators: the composition of the cluster (cluster mass, the mass of peduncles and berries in a cluster) and the composition of the berries (weight of 100 berries, weight of flesh, fruit skin and seeds in 100 berries) expressed in grams. As for the parameters of the chemical composition of the stum pH, sugar and acids content in the stum were investigated.

Results and discussion

Absolute and relative values of the parameters of the mechanical composition are different for different varieties of vines and for different growing conditions. Knowledge of mechanical composition of berries and cluster has a special practical importance in assessing the grapes as raw material for processing and consumption in fresh condition (*Blesić*, 2006).

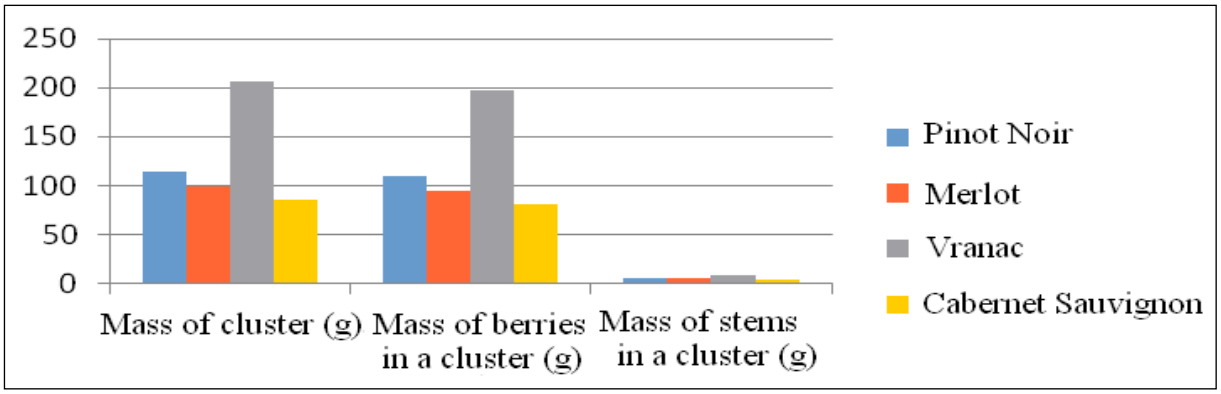
Mechanical composition of cluster. The relationship between certain parts of the cluster depends on the grape variety, the health condition of the grape, ecological conditions of cultivation and harvesting time. The values of certain parameters of the mechanical composition of the cluster of the tested varieties are presented in Table 1.

Table 1. - The values of the mechanical composition of the cluster of the tested varieties

Variety	Pinot Noir	Merlot	Vranac	Cabernet Sauvignon	LSD 0.05	LSD 0.01
Mass of cluster (g)	114.95	98.73	206.93	85.35	29.21	39.04
Mass of stems in a cluster (g)	5.58	4.85	8.87	3.78	1.27	1.70
Mass of berries in a cluster (g)	109.36	93.89	198.05	81.56	28.85	38.56

The mass of the cluster is a quite steady ampelographic property and it is defined by the biological characteristics of varieties of vines. Out of all tested varieties, Vranac variety had the highest cluster mass (206.93g) and Cabernet Sauvignon had the lowest (85.35g). Analysis of the significance of differences of the average cluster mass of the tested varieties shows that there is a highly significant difference in mass of the cluster between the varieties of Pinot Noir and Vranac, Merlot and Vranac, Cabernet Sauvignon and Vranac.

A significant statistical difference in terms of the mass of the cluster can be found between the varieties of Pinot Noir and Cabernet Sauvignon. Our results in terms of the mass of the cluster are consistent with the results of other authors (*Avramov et al*., 2003; *Pajović et al*., 2009; *Popović et al*. 2013), however, they deviate slightly from the results of *Vukosavljević et al*. (2011) and *Ranković- Vasić et al*. (2011).



Graph 1. - Mechanical composition of cluster

Mass of stems in a cluster. The results of our study (graph 1.) show that the highest mass of stems in a cluster is present in Vranac variety (8.87g) and the lowest is present in Cabernet Sauvignon variety (3.78g). In terms of weight of the stems, the studies of varieties showed highly significant statistical difference.

Only between the varieties of Pinot Noir and Merlot, as well as Cabernet Sauvignon and Merlot there was not any statistically significant difference. The results of our examinations are consistent with results of *Pajović et al*. (2009).

Mass of berries in a cluster. Since from the aspect of the use-value of the varieties it is better if the variety has a higher share of berries in the structure of the cluster, it can be concluded that out of the investigated varieties, Vranac variety had the best results (198.05g) (Table. 1).

The results of the mass of berries of the cluster in our study are consistent with the results of *Pajović et al.* (2009). In terms of mass of the berries in a cluster between Pinot Noir variety and Vranac, as well as Merlot and Vranac there is a statistically very significant difference, but between the other tested varieties there is not any statistically significant difference.

Mechanical composition of berries. Berry consists of an external layer – berry skin, flesh (mesocarp and endocarp) and seeds. The values of the parameters of the mechanical composition of berries in a cluster of tested vine varieties in conditions of Trebinje in 2013 are given in Table 2.

Table 2. - Values of mechanical composition of berries of the tested varieties

Variety	Pinot Noir	Merlot	Vranac	Cabernet Sauvignon	LSD 0.05	LSD 0.01
Mass of 100 berries (g)	120.65	126.32	187.79	100.99	9.97	15.10
Mass of flesh of 100 berries (g)	107.57	95.51	150.41	75.97	11.46	17.36
Mass of berry skin of 100 berries (g)	15.16	24.21	28.51	19.54	2.72	4.12
Mass of seeds of 100 berries (g)	5.09	5.59	8.87	5.48	1.71	2.82

Mass of 100 berries. The study results showed that Vranac variety had the highest mass of 100 berries (187.79g) and Cabernet Sauvignon had the lowest (100.99g). Analysis of the significance of differences of masses of 100 berries in tested varieties shows that there is a significant difference between the tested varieties. Only between varieties Pinot Noir and Merlot there are no statistically significant differences. The data obtained for the studied varieties is consistent with the results of *Pajović et al*. (2009).

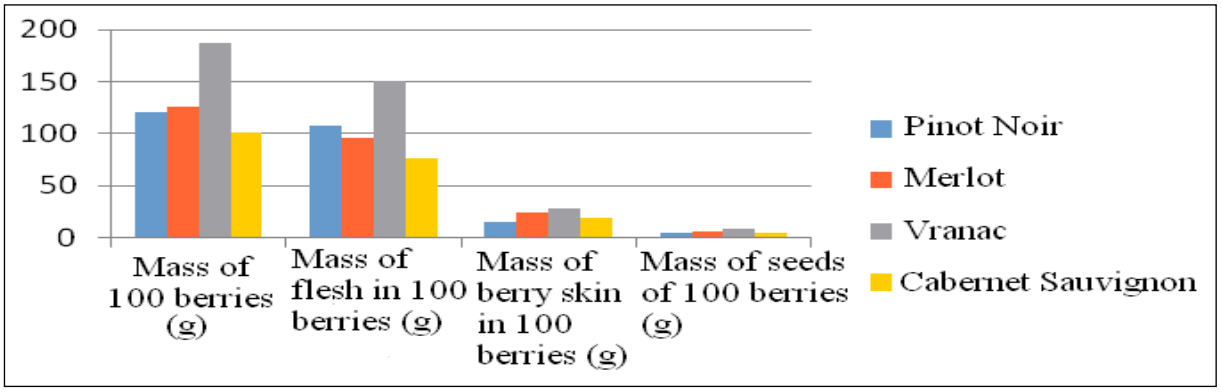
Mass of flesh in 100 berries. Fleshy part of berries (mesocarp) makes most of the berries. The results of the study of the flesh mass in 100 berries show that Vranac variety had the highest mass of flesh (150.41g) and Cabernet Sauvignon had the lowest (75.97g).

In terms of the flesh mass in 100 berries there is statistically highly significant difference among the tested varieties. Only between varieties Pinot Noir and Merlot the difference was statistically significant. Our findings are consistent with the values specified by *Pajović et al*. (2009).

Mass of berry skin in 100 berries. The thickness and elasticity of the skin depends on the variety and growing conditions. Vranac variety had the highest mass of berry skin in 100 berries (28.51g) variety Pinot Noir had the lowest mass (15.16 g) (table 2.). Analysis of the significance of differences in mass of berry skin in 100 berries of tested varieties shows that there is a statistically highly significant difference.

Mass of seeds in 100 berries. The study results showed that Vranac variety had the highest mass of seeds in 100 berries (8.87g) and Pinot Noir had the lowest (5.09g). A highly statistically significant difference can be seen between varieties Pinot Noir and Vranac, Merlot and Vranac and Cabernet Sauvignon and Vranac, while among other varieties there

were no statistically significant differences. The values for the mass of seeds in 100 berries cited by *Pajović et al*. (2009) are consistent with the results of our research.



Graph 2. - Mechanical composition of berries

Chemical composition of the stum is very complex. It varies dramatically, depending on the variety, environmental conditions, applied agricultural technology, degree of ripeness of the grapes, health status, etc. Out of parameters that are included in the chemical composition of the stum, in our study we included: sugar content, acid content and pH. The results of our study of the chemical composition of the stum in tested varieties are shown in Table 3.

Table 3. - Chemical composition of the stum in tested varieties

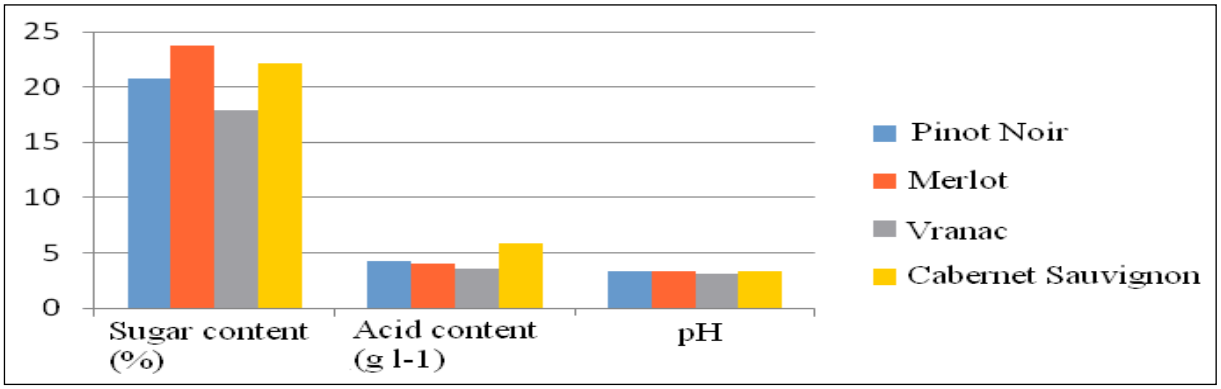
Variety	Pinot Noir	Merlot	Vranac	Cabernet Sauvignon
Sugar content (%)	20.80	23.70	17.90	22.20
Acid content (g l^{-1})	4.30	4.00	3.60	5.90
pH	3.35	3.37	3.08	3.29

The total amount of sugar in the stum varies and depends on the influence of variety, weather conditions during the ripening of grapes and applied agricultural technology. In most cases the sugar content of grape stum from noble vines of *Vitis vinifera* L. ranges between 160 and 250 g l-1 (16-25%). The results show that variety Merlot, had the highest sugar content in stum (23.7%), Pinot Noir had slightly lower sugar content (20.8%) and Cabernet Sauvignon (22.2%), and Vranac had the lowest sugar content out of all of them (17.9%). Our findings are consistent with the results of the author (*Cvetković et al*., 2000; *Mijović et al*., 2004; *Mijatović et al*., 2009; *Bešlić and Todić*, 2010), while *Stanković et al*., 2000, *Avramov et al*. 2003, *Vukosavljević et al*., 2011, *Popović et al*., 2013, report lower or higher values. In terms of sugar content in grape stum, we can see a significant impact of weather conditions and agricultural techniques applied to the tested varieties.

The amount of acid in ripe grapes varies in a very wide range, from 3 to 12 g l^{-1}, mostly 5-8 g l^{-1}, depending on the variety, environmental conditions, degree of ripeness of the grapes, etc. (*Nikolić*, 2012). The grapes which ripen in warmer climates will have less acid than those which ripen in colder climates.

The results of our study (Table 3) showed that the highest content of acid in stum had the Cabernet Sauvignon variety (5.9 g l^{-1}) and Vranac variety had the lowest (3.6 g l^{-1}). The obtained values are significantly lower compared to the results of other authors (*Avramov et al*., 2003; *Mijović et al*., 2004; *Mijatović et al*., 2009; *Bešlić and Todić*, 2010; *Vukosavljević et al*., 2011; *Popović et al*. 2013). The differences in values for a given feature can be interpreted

through the influence of different weather conditions during the ripening of grapes and applied agricultural technology.



Graph 3. – Chemical composition of the stum

The pH value of the must and wine mainly ranges between 2.7 and 3.9 (*Katalinić et al*. 2010). More acidic wines have a value below 3.5, while in the insufficiently acidic wines it moves up to 4. Vranac variety had the lowest pH value (3.08) (graph 3.) and Merlot had the highest (3.37). The pH value of the tested varieties is slightly lower compared to the results of *Pajović et al*. (2009) in Podgorica vineyards. However, it is in accordance with the values specified by the same authors in Skopje vineyards. Our findings are consistent with the results of *Bešlić and Todić* (2010) and *Yuste et al*. (2013).

Conclusions

1. Vranac veriety had the highest mass of cluster, and Cabernet Sauvignon had the lowest. Also, Vranac showed the best qualities regardin the mass of stems and berries in a cluster, while Cabernet Sauvignon had the lowest results for these qualities.
2. Mass of berries and mass of berry flesh in 100 berries was the highest in Vranac variety and it was the lowest in Cabernet Sauvignon variety. Vranac had the highest mass of berry skin and mass of seeds, and Pinot Noir had the lowest.
3. Sugar content in stum varied from 17,9% (Vranac) to 23,7% (Merlot). Vranac variety had the lowest content of acids, and Cabernet Sauvignon had the highest. Vranac had the lowest pH value and Merlot had the highest.

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EXAMINATION OF VIGOR OF DOMESTIC GENOTYPES BIRDSFOOT TREFOIL (Lotus corniculatus L.)

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Abstract

Seed aging is one of the main causes of reducing vigor, germination and emergence of Birdsfoot trefoil's seed. In this study, the vigor of domestic birdsfoot trefoil genotypes tested in field conditions. Seeds collected from the local population of 20 genotypes; 10 genotypes from 5 till 6 years old and 10 genotypes seed from 15-16 years old was examined. The seeds were stored in paper packaging in storage conditions. The viability of seeds represents a potential for formation of a new plant. The aim of this study is to determine the seed germination under field conditions and to obtain new plants capable for reproduction. Based on the results of field trials, can be concluded that germination for genotypes of seed from 15 till 16 years old has the high degree of variation (7-48%), while the genotypes of seed from 5-6 years old, interval of variation was lower (71-92%). Age of seeds had no effect on the production of green mass and seed yield. On the basis of the examination of the seed germination of local genotypes *Lotus corniculatus* L., it can be concluded that this plant species belongs to the group of plants with macrobiotic seed.

Keywords: *seed vigor, seed age, germination, sprouting.*

Introduction

The viability of seeds of birdsfoot trefoil is very important for the establishment of crop plants. Seeds of those genotypes with high vigor are recommended for sowing (*Artola et. al.,* 2003). In the opinion of numerous authors, *Lotus corniculatus* L. originates from the Mediterranean, but it is transmitted and widespread to South and North America, Australia and New Zealand. The greatest divergence of *Lotus corniculatus* L. appears in the region of Mediterranean (*Grant,* 1991), and includes the territory of Serbia and Bosnia and Herzegovina. Significance of birdsfoot trefoil for livestock development in mountainous regions is huge. The possibility of expanding *Lotus corniculatus* L., especially when mixed with herbs, is extremely high. One of the conditions for faster expansion of this kind is constantly improving existing varieties, the introduction of new breeding materials.

Mc Graw *et al*., (1986) are investigated the influence of latitude on the interaction of genotype - conditions in terms of seed yield *Lotus corniculatus* L. Investigations were carried out in three US states (Minnesota, Wisconsin and Missouri). The results indicate the powerful effects of abiotic environmental factors, among these three locations, which is why the authors recommend the selection of *Lotus corniculatus* L. variety and selection activities for each area. Winch *et al*., (1985) notes that the huge losses in yield due to seed dispersal.

Rincker (1980) examined the effect of keeping the seeds of legumes at low temperatures and their impact on the yield of green mass. Seeds of birdsfoot trefoil, alfalfa, red clover and alfalfa hybrids 15 years old, stored from the time of harvest at a temperature of -15 ° C and a relative humidity of 60 % have been sown. The green mass yield did not show significant differences between 15 years old seeds and fresh seeds, which means that at low temperatures, the seeds can be stored for many years. The condition is that the seed has good germination after harvest.

Smith *et al.*, (2009) and Beuselinck *et al*., (2005) are investigated the effect of seed origin from geographical and environmental distant populations due to the phenotype and genotype. They found regional differences in the size of the plants, growth, sexual maturity, the sheet size and fertility.

Ujević - Kovačević (1972) concluded that seed germination depends on a number of hereditary and external factors. The same authors found that the mass of 1000 seeds, plant development, environmental conditions, etc., as an important indicator of the quality of seeds depends on variety. Previous studies in breeding programs of birdsfoot trefoil emphasize the advantage of local populations in relation to supplies from other regions, the fact that acclimated ecotypes achieve better production results compared to the introduced populations and varieties (*Steiner et al*., 2001; *Sareen*, 2004; *Radic et al*., 2014).

There is a large number of population's birdsfoot trefoil that possesses considerable genetic variability in the agro-ecological conditions of Bosnia and Herzegovina (*Radić*, 2010). Vučković *et al.* (2007) tested in an experiment the ten populations birdsfoot trefoil from Serbia and Bosnia and Herzegovina on the basis of morphological, ecogeographically and chemical characteristics, have determined according to a number of morphological and chemical properties, that this population can serve as a valuable material for selection

Lotus corniculatus L., is the most important plant species of the genus *Lotus*, which has a high nutritional value and does not cause bloating at nutrition stock in the green state (*Beuselinck and Grant*, 1995).

The aim of this study is to determine germination of different ages of birdsfoot trefoil in field conditions and to obtain new plants capable for reproduction.

Materials and Methods

During the selection at pollinated plants a part of the seed is left in reserve to preserve a certain genotype. The longevity of seeds is different in different plant species, and depends on the genotype and the manner of storage. We need to distinguish terms of viable and vital seed for tests on seed germination. A viable means seed where the germ live and in which place life processes and vital seed is that which germinate in natural conditions.

In order to reduce the cost of seed multiplication is very important to know what the length of life of seeds, in order to preserve valuable selection material. To test was used seeds stored in storage conditions in the paper package. From seed left in the reserve during the preceding breeding cycle and taken the seed of those promising genotypes that showed a number of positive production traits. It is 20 genotypes of domestic origin of *Lotus corniculatus* L. obtained in a previous cycle of selection in natural populations from different locations throughout Bosnia and Herzegovina. The seeds of local genotypes 20 of birdsfoot trefoil are between 5 and 16 years old stored at room temperature sown in *Jeffi* containers filled with substrate. Testing germination was conducted in two groups. The first group consisted of ten genotypes seed from 15 till 16 years old end second group of ten seed from 5 till6 years old. The sowing was carried in containers with four replications of 100 seeds. The seed was planted in the substrate, and after sowing, the containers placed in the greenhouse. Water spraying is done every day. Number of emerged plants was determined after 10, 17 and 24 days. Counting of emerged seed was conducted in the same time periods, growth saplings are monitored per day and atypical plants were removed from each genotype. The plants are replaced in open space due to tempering after thirty days. Seedling growth was slow in the initial period of tempering but all the plants were vital and continued growth. When the plants have reached a height of 10 cm transplant was performed on experimental plots. Data on germination and formation of young plants were statistically analyzed and graphically presented. From each genotype data were taken, all the repetitions and represented as mean values , and then compared with each other. Correlations were identified as Pearson

correlation coefficients. Correlations were found between duration of storage of seed and germination, as well as between the time terms for determining germination.

Results and Discussion

The viability of seeds involves the possibility of formation of a new plant. It is conditioned by the amount of embryos and reserve materials. If embryo is well developed and fulfilled, it is the main condition for obtaining a strong saplings and normally developed plants (*Milošević,* 1987).

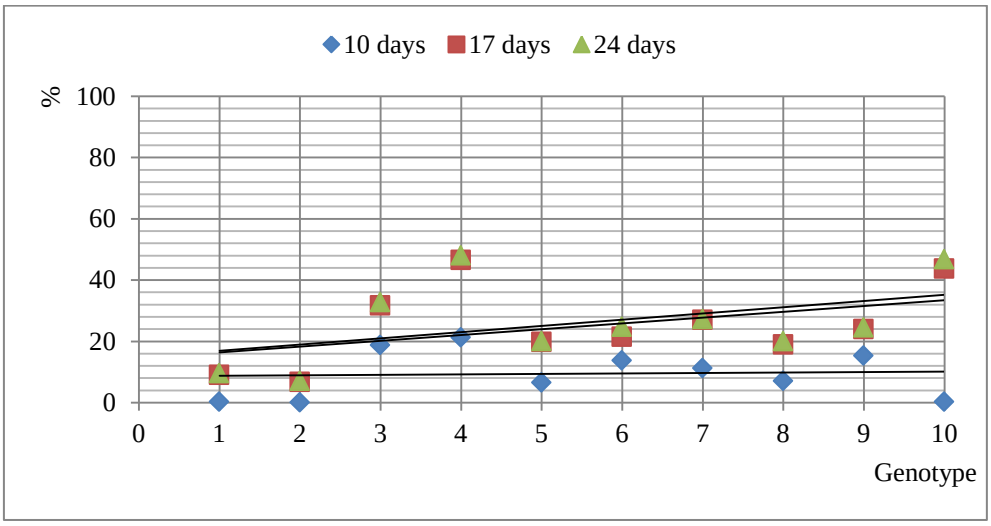

Figure 1. Germination seeds of genotypes of birdfoot trefoil from 15 till 16 years old.

Figure 1 shows the results of germination genotypes of Birdsfoot trefoil of 15-16 years old. Germination was in the interval from 0 to 21%, (in average of 10 %) in the first count after ten days from sowing In determining germination after 17 and 24 days were found small differences in germination in tested genotypes Observing the line of mean values of these tests can be concluded that they almost match. This fact leads us to the conclusion that most seeds germinate after 17 days. The total lowest germination after testing of these genotypes had genotype two (7 %) and the highest genotype four (48%). The average germination was 26% of genotypes of 15-16 years old.

For tested genotypes is determined the degree of multiple correlations between germination in three periods count (r = 0.55). The coefficient of partial correlation was the lowest among the first and third counting ($r_{1,3}$=0,50), and the highest between the second and third counting ($r_{2,3}$=0,99).

By observing the figure 2 in which the data are presented to germinate of seeds of ten local genotypes birdsfoot trefoil age 5-6 years old, it can be concluded that all have a high percentage of germination. Total germination ranged from 71 to 92%. These indicators tell us that the seeds birdsfoot trefoil kept in storage conditions can be used for productive purposes and up to six years after the harvest.

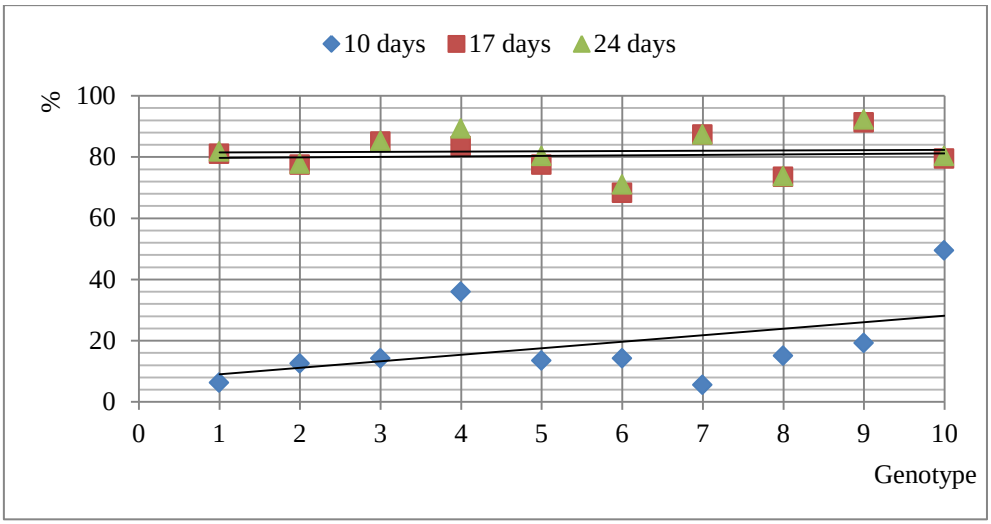

Figure 2. Germination of genotypes of birdsfoot trefoil from 5 till 6 years old.

The lower degree of correlations (r=0,38) is determined in relation to the seed which has been longer stored (r=0,55). Correlation was less between the first and third counting ($r_{1,3}$=0,13), while the correlation between the second and third counting ($r_{2,3}$=0,96) showed a high degree of interdependence as well as the first group of plants.

Observing germination at intervals of counting, shows that the much smaller difference in the first counting the genotypes of 15 to 16 and 5 to 6 years old whereas the difference in the second count was dramatically higher. Observing the seed depending on the age of seeds can be seen that in genotypes seed 15-16 years old have a wide degree of variation (7-48%), while genotypes whose seed was age 5-6 years old, variation interval was less (71-92%). The difference in germination after 17 and 24 days a small, the same as in the first ten genotypes in which the seed longer guarded. The biggest difference between the first and second counts in seed germination in seven genotype was found. In the third counting period, there is no significant difference in the percentage of germination in relation to the second count.

The seed that had a longer period of conservation has kept the germination of 7% (genotype 2) up to 48 % (genotype 4), and the seeds of genotypes 5-6 years old germinated from 71 % (genotype 2) to 92 % (genotype 9). Similar results obtained Artola *et al*. (2003). This indicator is very important from several aspects such as: biological, ecological, economic, and genetic and others. Seed viability of this plant species is a good predisposition for further work in the selection. Seeds of birdsfoot trefoil can be stored for many years at room temperature and will maintain the level of commercial viability standard. This means that the seed birdsfoot trefoil be marketed through six years of age, but it is necessary before sowing to carry out control germination.

Seed age had no influence on the formation of vegetative and generative organs (figure 3). By visual examination of saplings is not noticed an increased number of atypical plants. Morphometric parameters studied lines not showed differences that can be brought in connection with the age of the seed. Age of seed has no effect on seed production of green mass and seeds, it is important to keep the seed germination longer period of time, to be used in various purposes has been concluded. Similar results obtained Rincker (1980) when he sowed the seeds of birdsfoot trefoil, alfalfa, red clover and hybrid of 15 years old, but a precondition is good germination to seed after harvesting, must have a high germination.

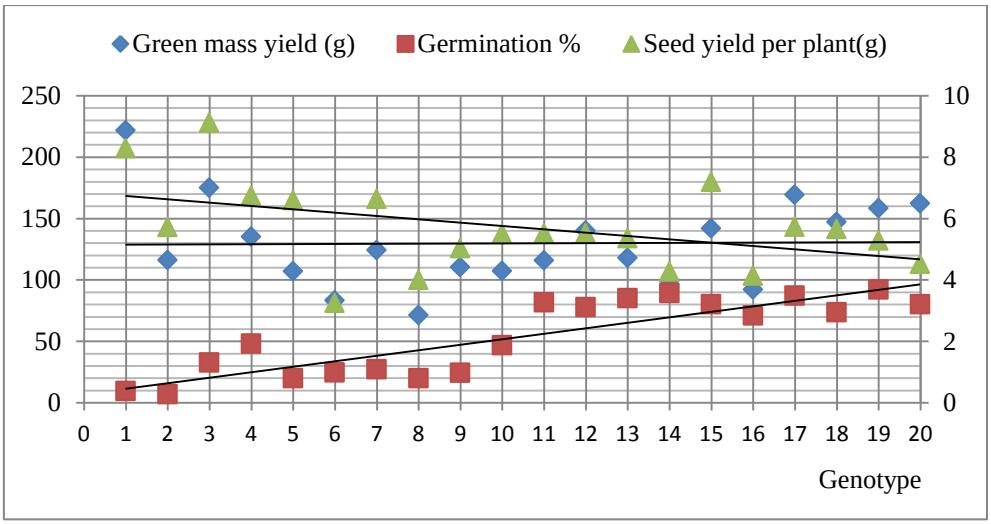

Figure 3. Interactional relationships between seed germination of the genotypes with the seeds production and biomass.

Based on the results of germination can be seen that longevity of seed of *Lotus corniculatus* L. belongs to the group of macrobiotic seeds (Milošević i Ćirović, 1994). Seed dormancy of *Lotus corniculatus* L. is quite high up 90% (McDonald, 1957), but age seed reduces the proportion of hard seed was found. On the basis of these studies we can conclude that with age seed, the percentage of dormant seeds decreases. At all lines of seed germination was good although he could not determine the number of hard seed. In laboratory conditions, hard seeds are counted in germinated seeds.

Conclusions

Seeds of genotypes with longer conservation of 15 to 16 years old kept the germination from 7% till 48%, and seed genotypes of 5 to 6 years old from 71% till 92%.

These results show that the seed of examined local genotypes of birdsfoot trefoil has a high germination, and about five years of age may be marketed.

Based on results of germination, the seeds longevity of *Lotus corniculatus* L. belongs to the group of plants with macrobiotic seed.

Age seeds of the examined genotypes of birdsfoot trefoil had no influence on the amount of green mass and seeds.

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DETERMINATION OF TOTAL PHENOLS AND ANTIOXIDATIVE ACTIVITY OF RED SWEET AND HOT PEPPER

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Abstract

In this paper the total phenols content and antioxidative activity of milled red (sweet and hot) pepper were determined. For extraction (90-95°C for 15 min), distilled water and methanol/water (30%) were used as solvents. The aim was to determine the total phenol content and antioxidant activity in sweet and hot pepper red spicy peppers using methanol and distilled water extracts.

The total phenols (TP) content (n=20), was determined spectrophotemetrically using Folin-Ciocalteu reagent at 600nm, and antioxidant activity by PFRAP method (with color reaction at 700nm).

The obtained data shows that highest (TP) content was found in sweet pepper 626.71 (in distilled water extract) and 368,27mg GA/100 of DW in 30% methanol extract. The average content of antioxidant activity of sweet spice pepper extract (distilled water) is 52.31mgGA /100g, while the value of hot pepper is 47.25mgGA /100g. The methanol extract mild red pepper is 9.18 mgGA/100g, while hot pepper is slightly higher (17.29 mgGA /100g).

It can be concluded that TP and the antioxidant activity of pepper was not affected by kinds of peppers (sweet or hot), but were affected by the solvent. The LSD test indicated that the difference in the antioxidant activity was significant, using two solvents (water and methanol/water). It showed that antioxidative activity was in correlation with total phenols content.

Key words: Red *pepper, phenol content, antioxidative activity, distilled water, methanol*

Introduction

Pepper (Capsicum annuum L.) is one of the most important vegetable crops in the world. There are many varieties of peppers that vary in colour, shape, size, flavour and degree of spiciness. Red pepper is used in all the kitchens around the world to improving the sensory properties of food, as a natural dye. It is rich in biologically active compounds including significant the phenol compounds, which are believed to have a positive effect on human health and are powerful antioxidants.

Phenol compounds are plant secondary metabolites and they possess a wide range of physiological functions as antioxidants: active anticancer, anti-inflammatory, anti-allergic, anti-microbial, and protect the cardiovascular system.

Phenolic acids make up about one third of the phenolic compounds present in plants, but they are present in free or bound form. In nature it is rarely found in free form, most often come in the conjugated forms and as esters (Macheix et al., 1990). Depending on the form of phenolic acids present, it is necessary to choose the extraction conditions that result in the least possible degree of degradation (Ross et al., 2009).

The antioxidant may be a substance present in low concentrations, which delays or prevents oxidation (Halliwell and Gutteridge, 1999). Because of the proven antioxidant activity peppers attributed to the presence of phenolic compounds, it has become a subject of research, and a very interesting for food industry. The level of antioxidants is different between the

cultivated species, mostly hot peppers are richer in antioxidants than fresh, and some writers claim that sweet peppers have a higher amount of phenolic compounds, and therefore higher antioxidant activity (Perucka and Materska, 2007). Pepper is rich in flavonoids, phenolic acids and carotenoids (Lee et al., 1995; Hasler, 1998). Since polyphenols are the most common: hydroxycinnamates, flavonols and flavones (Marin et al., 2004). Sweet peppers contain volatile components, fatty acids, carotenoids (5.8 g/g fresh weight), vitamins, proteins, fibers, minerals (Bosland and Votava, 2000; Materska and Perucka, 2005).
Due to the favourable impact of the consumption of peppers on human health, the goal of this work is to extract and determine the total phenol content and antioxidant activity in sweet and hot pepper red spicy peppers.

Materials and methods

Packages of sweet and hot red spice pepper were used for research. A total of 10 packages of sweet spice pepper and 10 packages of hot. Packages were purchased in shopping centers in Sarajevo Canton, Bosnia and Herzegovina. Samples were stored in dry, dark and cool places before analysis.

• Extraction with distilled water: Samples were homogenized in fine powder and about 2 g of the powder was extracted with 40 ml of hot distilled water at 90-95°C for 15 min (using reflux). The resulting extract was filtered into a volumetric flask of 50 ml ,with the addition of distilled water to the mark.

• Extraction with 30% methanol: Sample of pepper (2g), added with 40 mL of (30:70) of methanol preheated to 50 ° C. The homogenized sample was transferred to a flask, with reflux condenser, wherein the extraction is carried out for 15 minutes at a temperature of about 90°C the extract was filtered in a 50 ml flask. The flask was amended solvent to stamps.

Determination of total phenols content

The total phenols content was measured using the Folin Ciocalteu reagens (FC), by a method based on colored reaction of phenols with FC (Ough and Amerine,1988), with slight modification. The absorbance of resulting blue color was measured at 600 nm (Shimadzu 2200 UV-VIS spectrophotometer). Quantification was done with respect to the standard curve of gallic acid (300 mg/L). The results were expressed as gallic acid (GA) miligram per 100g of dry weight (DW).

Determination of antioxidant activity (pFRAP method)

Determination of antioxidant activity was performed by spectrophotometry using pFRAP (potassium ferric antioxidant power) method according Meng et al., (2011) and Jayaprakasha et al., (2003), with slight modigications. Components with antioxidant activity react with $K_3[Fe(CN)_{6]}$ and translate it into a form of $K_4[Fe(CN)_6]$. Reaction with $FeCl_3$, is resulted by production of blue colored complex with maximum absorbance at 700 nm. The antioxidant activity is expressed as mgGA/100g.
All measurements were performed in triplicate. The results were evaluated statistically in excel by two–way analysis of variance (ANOVA). To compare the average value LSD test was used.

Results and discussion

Data on total phenol content and antioxidant activity in sweet and hot pepper spicy pepper, obtained by laboratory research are presented in the tables1 and 2.
It was found that the total phenol content of spice pepper no significant impact (sweet, hot) ($F_{calc.} = 0.004245 < F_{crit} = 4.493998$), but is affected by the influence of the type of solvent that was used in preparing the extract (distilled water / methanol: water) ($F_{calc.} = 8.357656 > F_{crit} = 4.493998$), while a significant impact of their interaction has not been determined (F_{calc}=

0.316276 <F_{crit} = 4.493998). The extract of sweet pepper (n=5), which is the solvent distilled water containing significantly higher average amount of total phenols (626.7048), the extract from which the solvent is 30% methanol (368.2711 mgGA /100 g).
Greatest amount of phenol in the distilled water extract was in the sample P_{13} (835.1847), while the least amount of phenol had a P_{12} (463.3256 mgGA/100g). The extracts of the samples with 30% methanol is recorded twice reduced the average amount of total phenols, so that the highest concentration of total phenolic compounds contained in the sample an amount was 729.8395 (P_5), while the minimum amount of phenol was observed in the P_4 (196, 3762 mgGA /100g).
The results showed that in the fresh spice pepper greater extraction capacity is achieved by using distilled water. Compared to distilled water, 30% methanol solution proved to be less efficient in the solvent extraction of total phenols. The results of total phenols in hot spicy red pepper have shown that there are significant differences between the content of total phenols in the spice pepper extracts obtained by methanol extract according to distilled water

Table1: The average of total phenols content in peppers in (30% methanol) and distilled water

Methanol 30%		**Samples**	**Average**	**Mean (mgGA/100g)**	**St. dev**	**Distilled water**		**Samples**	**Average**	**Mean (mgGA/100g)**	**St. dev**
	Sweet pepper	P1	368,2711	237,6636	0,4823		**Sweet pepper**	P11	626,7048	611,9887	0,7515
		P2		303,1821	1,4048			P12		463,3256	0,5037
		P3		374,294	0,1845			P13		835,1847	0,5566
		P4		196,3762	0,2942			P14		710,7362	1,0907
		P5		729,8395	5,0971			P15		512,2889	0,3225
	Hot pepper	P6	415,2334	429,6863	0,0941		**Hot pepper**	P16	589,4942	760,2379	0,3375
		P7		538,2125	0,3776			P17		319,4117	0,24104
		P8		247,5392	0,2178			P18		670,1507	0,01250
		P9		331,9597	0,3264			P19		555,0276	0,2454
		P10		528,7692	0,6517			P20		642,6432	0,08007

Table 2: The average antioxidative activity peppers in (30% methanol) and distilled water

Methanol 30%		Samples	Average	Mean (mgGA/100g)	St.dev	Distilled water		Samples	Average	Mean (mgGA/100g)	St.dev
	Sweet pepper	P1	9,18226	8,8106	0,0739		Sweet pepper	P11	52,30522	42,0663	0,2134
		P2		4,8587	0,0625			P12		63,7299	0,3305
		P3		9,1704	0,0523			P13		70,4102	0,0591
		P4		6,1995	0,0693			P14		52,6473	0,0143
		P5		16,8723	0,0733			P15		32,6706	0,0694
	Hot pepper	P6	17,29646	21,7804	0,0578		Hot pepper	P16	47,2516	49,6726	0,0301
		P7		6,8941	0,0602			P17		44,2129	4,9124
		P8		18,3723	0,0463			P18		55,4646	0,0657
		P9		18,0466	0,0226			P19		44,8658	0,0636
		P10		21,3890	0,0651			P20		45,0421	0,0457

The average total phenols in the water extracts (n=5) was higher (589.4942) in relation to the methanol (n=5) extract (415.2334 mgGA/100g). The maximum total phenols content was in spicy pepper with using of distilled water as solvent 760.2379 mgGA/100g (P_{16}), while the minimum content was observed in the sample P_{17} to the value of 319.4117 mgGA/100g. At least registered concentration at 30% methanol extraction is reached with the sample P_8 (247.5392), and the largest concentration of phenol was observed in the sample P_7 (538.2125 mgGA /100g). In hot spicy peppers, better extraction capacity is achieved using distilled water; it was evident that the extraction with distilled water greater proportion of total phenols in comparison to a methanol extract was determined.

The average value of antioxidant activity of the extract in distilled water was greater in sweet peppers (52.30522), and lower in hot pepper (47.25156 mgGA/100g). In spicy peppers harvested with 30% methanol, the antioxidant activity was hot pepper with an average value (17.29646), while the sweet pepper contained lower average antioxidant activity were 9.18226 mgGA /100g. It was determined that there are no significant differences in types of pepper (sweet and hot), but there are species with a solvent (distilled water / methanol). Materska and Perucka (2005*)* were showed that antioxidative activity of pepper extract was depended mainly on phenolic compounds.

It has been shown that total phenolic compounds varies between periods of production of pepper, so that the measured values of 675 and 136 mgGA /100g, depending on the time of sowing and harvesting. Studies have shown that the highest average total phenol content was recorded in paprika produced in the month of November (1360 mgGA / 100g), while the Indian peppers recorded the total amount of phenol in the value of 500mgGA / 100g (Tripathi and Mishra, 2009). Flamingo peppers contain a total amount of phenol, about 852 mgGA / 100g (Deepa et al., 2007).

The total amount of phenol in the hot Mexican peppers was (20-782 mgGA /100g), according to Lee et al., (1995), and Menichini et al., (2009).

According to Kumar et al., (2010) the content total phenols in fresh pepper was 266mg/g fresh weighs.

Conclusions

Results, statistical analysis showed that the impact of antioxidant activityand total phenols is not significant species pepper (sweet, hot), but is determined by the influence of the type of solvent that was used in preparing the extract (distilled water / methanol: water). The average antioxidant activity of sweet spice pepper extract in distilled water is 52.30522, while the value of spicy pepper is 47.25156 mgGA/100g. The average antioxidant activity of the methanol extract with mild red pepper is 9.18226, with hot pepper is slightly higher at 17.29646 mgGA /100g.

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EFFECT OF THE DATE OF APPLICATION OF HERBICIDES ON THE PRODUCTIVITY OF COMMON WINTER WHEAT

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Abstract

The effect of the herbicides Derby Super VG, Granstar 75 DF, Lintur 70 VG, Secator OD, Mustang 306.25 SK, Palace 75 VG and Husar Max OD, applied at different phenophases, on the productivity of two major common winter wheat varieties (Enola and Aglika) was investigated. Five-factor dispersion analysis was used to evaluate the effect of the factors genotype, climatic conditions, sowing date, herbicides and phenophase of treatment. As a result from the investigations carried out on the date of treatment with the herbicides and its influence on the productivity of common winter wheat it was found out that best results were obtained after using the herbicides at tillering stage. Regardless of the cultivar or the sowing date, lowest losses of wheat were observed after treatment with the preparations Derby Super VG and Secator OD.

The application of the herbicides at the end of tillering allowed wheat to realized its highest productivity regardless of the sowing date or the cultivar used. The application of the herbicides at phenophase first-third node was related to lower efficiency of some of them as Granstar 75DF, Palace 75WG and Husar Max OD. After the use of Derby super VG and Secator OD at this phenophase, no significant decrease was found neither in the efficiency against weeds nor in wheat productivity. The use of herbicides at phenophase emergence of flag leaf was accompanied with further decrease of wheat productivity. On the one hand, this was due to the growth of weeds and the lower efficiency of the used herbicides, and on the other – to increasing of toxicity on wheat caused by some herbicides. Due to the higher toxicity of the herbicides Lintur 70VG and Mustang 306.25 CK on wheat, greatest yield decrease was registered after using them at this phenophase.

The influence of factors “phase of treatment“ and “climatic conditions” was proved with the highest statistical significance, and the factor “date of sowing” - with the lowest one.

Key words: winter wheat , herbicides , productivity

Introduction

A main method for control of weeds in the cereal crops on a whole area is the chemical method, or the use of herbicides. Barros et al., (2007) reported that Akurat 60VG (3.0 %) and Grodil ultra VG (0. 6%) were used against annual broad-leaved weeds: *Galium aparine (L.)(* cleavers), *Anagallis arvensis (L.)*(scarlet pimpernel), *Lactuca seriolla (L.)*(prickly lettuce), *Daucus carota (L.)*(wild carrot), *Lamium amplexicaule (L.)* (henbit dead-nettle), *Scandix pectin- veneris (L.)*(shepherd`s needle). After later application of the herbicides (phenophase of weeds 5^{th} – 6^{th} pair of leaves and phenophase of the crop booting), lower yields were measured. Akurat 60VG (3.0 %) provided satisfactory control when applied at booting stage for control of weeds, without decreasing yield.

The investigation showed that after treatment at stage end of booting of wheat, best results were obtained from Banvel K, which increasing yield with 19.0 %, followed by 2,4DP with 17.9%. Lowest was the increase after 2,4D - 10.1% . The active substance dicamba in the composition of the herbicide Lintur 70VG decreased the yield from barley with an average of

5.0 %, when applied at stage end of tillering, and at later treatment at stage visible 1st and 2nd node – with averagely 32.0 % .
The use of Palace 75WG and Husar Max VG at phenophase tillering lead to higher yields in comparison to the application of the herbicides against broad-leaved weeds Derby super VG, Weedmaster 464SL and Laren 60VP. High yield from wheat was obtained after treatment with Derby super 175СК (5 ml/da) and Puma super 7,5EV(100ml/da) at phenophase full tillering of the crop .
The aim of the research was to determine the effect of the date of application of a set of herbicides on the productivity of common winter wheat cultivars Aglika and Enola.

Material and methods

The research was carried out during 2012 – 2014 at Dobrudzha Agricultural Institute – General Toshevo (Bulgaria). The field experiment was designed according to the block method in four replications, with size of the experimental plot 15 m^2. Two check variants were involved: K_1 – weed-free check manually weeded till phenophase heading of wheat, and K_2 – check weeded till the end of the crop's growing season.
The following herbicides were used: Derby super VG (florasulam+aminopyralid- potassium) – 3.3 g/ha, Granstar 75DF (tribenuron-metil) - 15 g/ha, Lintur 70VG (triasulfuron+dicamba) - 150g/ha, Secator OD (amidosulfuron+iodosulfuron) - 100 ml/ha, Mustang 306.25СК (florasulam+2.4-D ester) - 800ml/ha, Palace 75WG+oil (pyroxulam) - 250+1000 g/ml/ha and Husar Max OD (mesosulfuron+iodosulfuron+antidot) - 1000 ml/ha, The preparations were applied at three stages of common winter wheat cultivars Aglika and Enola (end of tillering, first – third node, emergence of flag leaf: stages 29, 32 and 37 according to Zadoks et al., (1974)).
Before planning of wheat, artificial weed infection background was created with the most widely distributed weeds in the region of Dobrudzha Agricultural Institute – General Toshevo. These were the annual broad-leaved weeds ivy-leaved speedwell, wild mustard, cleavers, chamomile types, field chamomile, royal knight's spur, and the perennial broad-leaved weeds creeping thistle and field bindweed.
Weed density was determined per unit area quantitatively by species from ¼ of a sampling frame, in four replications prior to the application of the herbicides.
The herbicide efficiency was determined 25-30 days after the use of the preparations for each species by qualtity and by weight from ¼ of a sampling frame, the weeds being in dry condition. The effect was evaluated according to a 9-degree scale of the EUROPIAN WEED RESEARCH SOCIETY for reading of the herbicidal activity and selectivity, 1 corresponding to 100 % herbicidal effect without symptoms of phytotoxicity on the cultural plants, and 9 – to 29.9 % - 0 % effect of the herbicide and total perishing of the plants.
Five-factor dispersion analysis was applied to determine the effect of the factor date of herbicide application on the productivity, the absolute and the test weight. Data were analyzed by the statistical package SPSS 19.0.

Results and discussion

The application of the herbicides at the end of tillering allowed wheat to realized its highest productivity regardless of the sowing date or the cultivar used. Averaged for the period of testing, the highest yield of 648.5 kg/da was obtained from the weed-free check at the optimal sowing date for wheat. The use of herbicides at this date allowed the realization of a yield within the range 93.2 – 96.0 % from the clean check. This was due to the crop, cultivated free from weeds. According to the weeded check (K_2), there was exceeding of 133.3 to 137.2% for the respective herbicides.

The sowing delay reduced wheat productivity of wheat. A yield of 608.2 kg/da was obtained, with 40.3 kg/da lower, than the yield obtained after sowing within the optimal dates of a weed-free crop. The application of the herbicides contributed to exceeding of yield of 131.1 to 134.4% from the yield obtained from the weeded check.

The application of the herbicides at phenophase first-third node was related to lower efficiency of some of them (Granstar 75DF, Palace 75WG and Husar Max OD). Concerning others (Mustang 306.25CK and especially Lintur 70VG), 28 to 56 days after their use, symptoms of phytotoxicity were found on the wheat plants in the form of anthocyanine coloration. The occurrence of phytotoxicity was more expressed after the use of Lintur 70VG. After the use of Derby super VG and Secator OD at this phenophase, no significant decrease was found neither in the efficiency against weeds nor in wheat productivity.

The use of herbicides at phenophase emergence of flag leaf was accompanied with further decrease of wheat productivity. On the one hand, this was due to the growth of weeds and the lower efficiency of the used herbicides, and on the other – to increasing of toxicity on wheat caused by some herbicides.

Regardless of the sowing date, highest yield from cultivar Aglika was obtained after the use of Derby super VG and Secator. The exceeding of the yield according to the weeded check varied from 124.8 to 125.5% after sowing within the optimal dates and from 134.0 to 134.7% after late sowing. It is necessary to point out that at this phenophase, incomplete destruction was also observed after the use of the above two products. The lower efficiency of Granstar 75 DF, Palace 75WG and Husar Max OD at this phenophase was better expressed than the efficiency at phenophase 1^{st} – 3^{rd} node. This reflected on yield. After both sowing dates, higher yield was obtained according to the weeded check, which varied from 110.2 to 115.6 % after the optimal sowing date and from 121.4 to 122.3 % after the late sowing date.

Due to the higher toxicity of the herbicides Lintur 70VG and Mustang 306.25 CK on wheat, greatest yield decrease was registered after using them at this phenophase. Regardless of the sowing date of cultivar Aglika, yield was obtained with 93.6 to 94.4% lower than the yield from the weeded check after the application of Lintur 70VG.

The means of squares of indices yield, absolute weight and test weight are pointed in table 1, also the interactions between the studied factors. By the index “yield” was proved with statistical significance p = 0.001, the influence of the factors “phases of treatment” and “climatic conditions “. The conditions during the years was the factor with highest part of the variation (40 %) by this character. The sowing date was with lowest (10%).

The statistical significance of the factors “herbicides” and “genotypes” were proved with p=0.01, also the interaction between the factors “genotypes” and “climatic conditions”. By the character “absolute weight” with highest statistical significance were proved the factors “phase of treatment“, “ecological conditions” and “genotypes“. By the index test weight all four factors were proved with statistical significance p=0.001 except the factor “date of sowing “.

Table 1. Mean of squares of studied factors .

Indices	Yield	Absolute weight	Test weight	df
H	3441.6 **	481.3 **	266.4 ***	8
F	4633.4 ***	571.4 ***	329.7 ***	2
D	2625.3 *	186.4	165.3 *	1
Y	10328.1 ***	1744.9 ***	588.1 ***	2
G	4158.8 **	1266.3 ***	388.6 ***	1
H x F	3752.4 **	435.8 **	199.4 **	16

H x D	1428.7	131.1	97.3	8
H x Y	2196.4 *	326.2 *	152.3 *	16
F x D	1236.8	99.5	88.9	2
F x Y	2743.2 *	391.4 *	168.7*	4
F x G	2345.1 *	386.2 *	155.4*	2
H x G	2638.4 *	247.6 *	162.4 *	8
D x Y	958.3	176.3	59.3	2
D x G	795.4	188.1	102.7	1
Y x G	3988.3 **	469.6 **	212.7 **	2
H x D x F x Y x G	1326.1	129.7	112.8	16
Error	459.4	56.3	29.8	122

H – herbicides, F – phase D – date of sowing, Y – yields , G – genotypes

In table 2 are presented the results of the five factors ANOVA for the structural elements of the “yield”. The index “plant’s height” for both varieties was estimated with highest values after the treatment in phenophase “end of tillering” of the crop. With the progress in this phenophase of the wheat, the values of this character decreased and the reason was the stronger influence of the weeds. The differences between the variants of treatments were not essential. According the character “plant’s height”, the following factors-“phenophases of treatment” , “herbicides” and “climatic conditions” were proved with statistical significance p=0.001. The indices “spike’s length” and “spikelets number” were genetically connected. The action of the herbicides by these characters was not essential. They ranged within narrow borders of both varieties. It was established, that the influence of the factor “ecological conditions” was the highest statistical significant one among the characters “spike’s length “and “spikelets number “.

The indices “spikelets number” and “grain’s weight” were the most variable. They decreased with delay in the term of herbicides application. The most essential decreasing of these indices was obtained by using of Lintur 70 BG and Mustang 306.25CK in phenophases “1st – 3rd node” and „emergence of flag leaf ”. This was due to the presence of sterility spikes, because of herbicides Lintur 70BG and Mustang 306.25.CK influence.

Table 2 . Means of squares of the structural elements.

Indices	Plant’s height	Spike‘s lenght	Spikelets number	Grain‘s number		Grain‘s weight	df
H	7428.5 ***	229.7 *	395.1 **	1122.1 **		0.926 *	8
F	5682.4 ***	399.9 **	442.3 **	1462.5 **		1,388 **	2
D	2848.9 *	215.3 *	142.3	653.2		0.603	1
Y	12645.4 ***	629.6 ***	861.5 ***	2867.7 ***		1.548 **	2
G	3746.8 *	407.5 **	322.6 *	1266.6 **		1.251 **	1
H x F	4428.5 **	348.1 **	296.4 *	625.1		0.887 *	16
H x D	6933.4 ***	257.4 *	471.6 **	924.3 *		0.624	8
H x Y	3199.2 *	122.1	299.3 *	872.5 *		0.358	16
F x D	2733.7 *	266.9 *	157.3	564.2		0.427	2
F x Y	3962.1 **	382.2 **	188.4	472.2		0.334	4
F x G	2762.1 *	288.3 *	177.2	386.1		0.294	2
H x G	2638.4 *	247.6 *	263.8 *	508.1		0.439	8

D x Y	2866.1 *	312.5 **	317.9 *	637.6		0.525	2
D x G	3022.5 *	269.4 *	199.2	482.2		0.882 *	1
Y x G	4691.3 **	422.1 **	283.6 *	1094.6 **		1.116 **	2
HxDxF x YxG	1877.4	194.6	156.3	825.3 *		0.392	16
Error	533.4	42.8	69.5	156.8		0.18	122

H – herbicides, F – phase D – date of sowing, Y – yields , G – genotypes

Conclusions

The applying of herbicides at the phenophase “end of tillering” permitted the wheat varieties to realize the highest productivity irrespective of the used variety or the term of sowing.

The influence of factors “phase of treatment“ and “climatic conditions” was proved with the highest statistical significance, and the factor “date of sowing” - with the lowest one.

The interaction of the factors “genotypes” and “climatic conditions” was statistical significant for all studied indices.

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RESEARCH ON SOME GRAIN QUALITY CHARACTERISTICS OF TURKISH AND ITALIAN RICE VARIETIES GROWN IN BULGARIA

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Abstract

A field experiment was carried out in the Saedinenie region, Plovdiv municipality, Bulgaria in the period 2013-2014. The basic grain quality characteristics of rice were studied within two Turkish (Osmanchik 97 – standard for Bulgaria, and Gala) and four Italian (Lince, Puma, Cameo and Brio) rice varieties. The following indicators were recorded: mass of 1000 grains, hectoliter mass and some biochemical indicators such as: starch content, crude protein and crude fats – in % of absolute dry substance. The sowing took place in the period 13-16 May involving the split plot design in four repetitions over an area of 14,85 m^2 at a sowing rate of 600 k.s./m^2. Rice was previously grown in the same area. The applied fertilization rate was $N_7P_{18.}$ The experiment was performed in conformity with an approved Bulgarian growing technology – the flooding irrigation regime. The statistical processing of experimental data was carried out via SPSS V.9.0 for Microsoft Windows.

On the average, it was defined that varieties Cameo – 37,11 g/1000 grains and Osmanchik 97 – 34,35g/1000 grains were outlined for largest grains within the stated period. The smallest grain size was observed with the Brio variety. The grain hectoliter mass of the tested varieties showed close values – between 43,25 kg (Cameo) and 44,60 kg (Brio). Only the Puma variety stood out with higher mass (48,88 kg).

For the period, the Turkish varieties Gala (8,80%) and Osmanchik 97 (8,30%) registered highest protein content on the average under Bulgarian conditions, followed by the Italian varieties Cameo (8,39%) and Lince (8,28%). Osmanchik 97 accumulated highest quantity of starch – 78,98%, followed by Brio – 76,70% and Linche – 76,69%. Crude fats stayed within the range from 3,42 (Brio) to 3,87% (Osmanchik 97).

Keywords: *rice, protein, starch, fats, grain quality characteristics*

Introduction

Rice quality is not always that easy to define as it depends on the consumers and its final envisaged application. It is the only cereal with whole edible grain thus making its quality much more important than that of any other cereal (Hossain *et al.*, 2009).

Traditionally, rice breeders concentrate on high yield and disease resistance. However, based on the recent research of 11 major rice-producing countries, authors such as Juliano and Duff (1991) have come to the conclusion that breeding is being redirected towards searching for quality characteristics targeted at increasing grain nutritional value.

Protein and starch content are the two dominant factors defining rice quality. Rice is an important source for protein, providing more than 50% of the total protein consumption in some countries. Most factors for its variation are related to the growing conditions (sun radiation and temperature in the grain-maturity period), as well as to the growing technology (stem density, nitrogen fertilization norm and time limit, irrigation regime and weed control). Negative correlation exists between protein content and rice yield, which is usually weak and depends rather on the growing conditions than on the genotype (Gomez, 1979).

Bulgarian rice producers have oriented their efforts towards the search for high-production potential varieties, fast adapting to the conditions in Bulgaria, and yet, with high-quality grain

relevant to the taste preferences of the Bulgarian consumer. The varieties produced in the Northern Mediterranean Region, for example Italian varieties, are considered to be of interest for being more adaptive and with high-production potential. The research carried out by Angelova, S. (1999), for example, defined that the Italian varieties Alfa and Artiglio stand out with higher yield and higher crude protein content in comparison with the Russian Krasnodarski 424 variety (used earlier as standard). In other studies carried out by Bulgarian authors, biochemical characteristics of introduced varieties was performed as well (Tosheva and Kamishev, 2013; Todorov and Angelova, 1998). Italian varieties are subject to other researchers in the Balkan Peninsula Region (Ilieva *at al*., 2000; 2013)

The aim of the present survey is to study the genotype influence on some physical and biochemical grain qualities of rice grown under the conditions of South Bulgaria.

Materials and Methods

A two-factor field experiment involving the split plot design was carried out in the Saedinenie region, Plovdiv municipality, Bulgaria in the period 2013-2014. The genotype was the basic studied factor (of the whole plots) in the experiment. Six induced varieties were tested – 2 Turkish and 4 Italian, using the Osmanchik 97 Turkish variety as standard. The experiment was performed after rice as a forerunner, in 4 repetitions with reported yield plot area of 14,85 m^2.

The 1000 grain mass was determined by the adopted technology in conformity with the Bulgarian State Standards (BDS). It was reported via mass measuring (g) of two samples of 500 grains (paddy rice) for each variety, from each repetition and for each experimental year.

The hectoliter mass (kg/100 l grain (paddy rice) was measured according to the adopted technology, in conformity with BDS.

The lab analyses were carried out in the Central Laboratory for Research at Agricultural University – Plovdiv. The analysis regarding the presence of raw protein was implemented under BDS 13490, for fats – under BDS 3412 established for wheat cultivars. The amount of starch content was measured with the polygraphic method.

The statistical processing of the tested data was performed via SPSS V.9.0 for Microsoft Windows. The proof for differences was reflected at significance level P 5% (Fowler and Cohen, 1992).

The experiment was carried out on fluvisol with humus content under 2,5%. The total cumulative nitrogen for both experimental years was between 10,81 and 11,50 mg/ 1000 g soil, which qualified the soil as poor, whereas with reference to cumulative phosphorus and potassium content, the soil was well supplied. The reported pH qualified the soil as acid (PH 5,81 to 5,18).

On the whole, the reported weather conditions for the vegetation year 2013 were favourable for rice growing (Fig. 1 and 2). Critical deviations from the basic climate affecting factors were not indicated during each crop development phenophase. The high after-sowing temperatures set up prerequisites for fast germination and intensive increase of plant height. The average temperatures from May to October were from +0,2°C to +3,8°C higher compared with the long-term period. This contributed to: accelerating the plant growth and development; forming a greater number of productive panicle elements; shortening the vegetation period.

The rainfall during the almost entire vegetation in 2013 was lower compared with the long-term period (Fig. 2). In August, during the flowering time, the rainfall was 32,7 mm lower than the long-term period, whilst normal pollination requires moderate and equally allocated showers.

The conditions in 2014 extremely differed from those in 2013. The average twenty-four-hour temperature results for the first two months of the vegetation period (May – June) showed that

at this stage rice was in more adverse conditions compared with the previous year. In May temperatures were considerably lower (by 2.7°C) than those in the long-term period and caused a negative effect on the germination length and preliminary development. Comparatively close temperature levels to those in the long-term period were measured from tillering to full-maturity, but they were between 0.5°C and 1.2°C lower in comparison with 2013.

The average monthly rainfall during the whole vegetation period in 2014 was drastically higher compared with the long-term period (Fig.2) and 2013. The registered heavy rainfall was an unfavourable prerequisite for rice growth as it changed the regulated water regime and cell microclimate. The amount of rainfall was lower only in August with registered temperature of 35.6°C, which did not favors normal rice flowering.

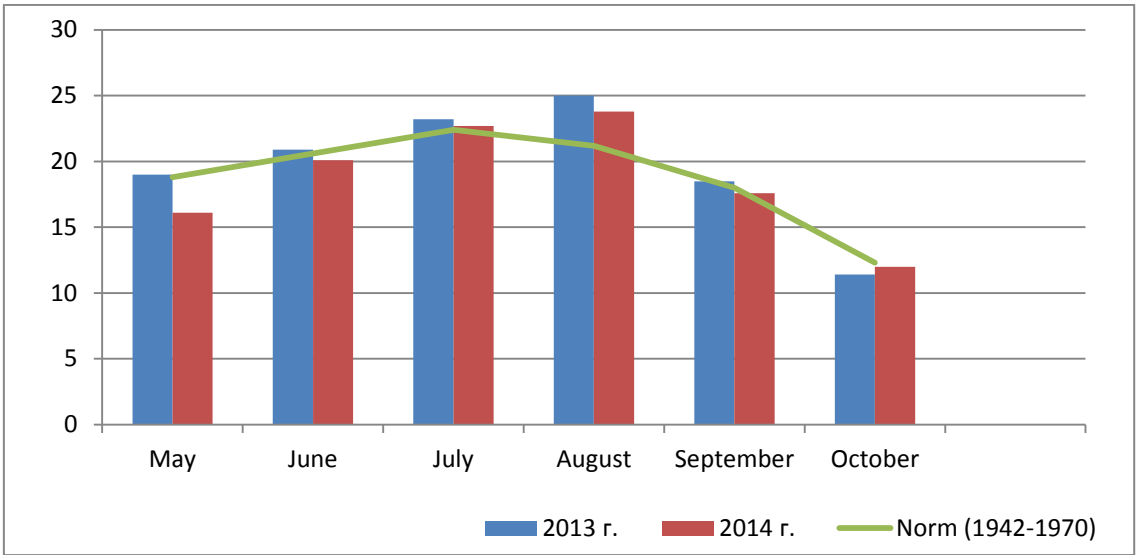

Figure 1. Average monthly temperatures (°C) for the research period, 2013-2014

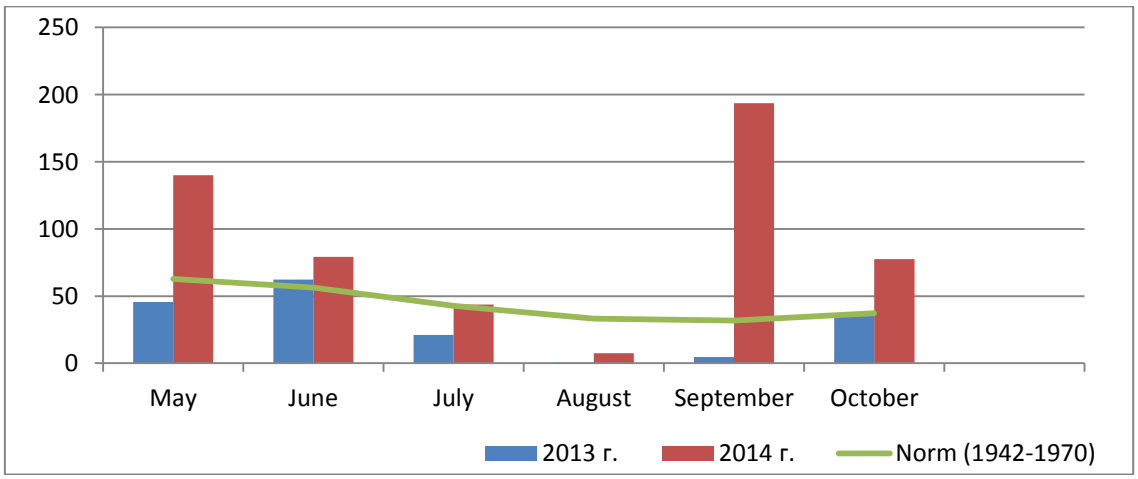

Figure 2. Total sum of rainfall (mm) for the research period 2013-2014

Results and Discussion

The 1000 grain mass is an indicator influenced by vegetation factors and the applied agrotechnics in rice growing. The tested varieties particularly differ by this indicator under the conditions of the performed experiment in 2013. The Cameo variety stands out with largest grain, overweighing the standard (Osmanchik 97) by 14,26%. It forms 37,90 g/1000 grain mass which statistically overweighs the indicators of the other varieties. Next comes Osmanchik 97, Gala and Puma with minimum difference – of about 2% and grain mass from 32,96 to 33,67 g. The Lince variety accumulates 32,52 g 1000 grain mass which is about 2% below standard. The Brio variety has got proven lowest indicator value of 29,31 g.

The 1000 grain mass of the tested genotypes in 2014 showed close values, fluctuating between 30,25 g for Brio to 36,32 g for Cameo. Osmanchik 97 also formed large grain - 35,52 g which drew it closer to Cameo while all the rest were with 32-33 g 1000 grain mass.

The summarized tendencies of the research for both years indicate that the Italian Cameo variety stood out with highest proven values by this indicator (mean 37,11 g). Its 1000 grain mass overweighed the Turkish Osmanchik 97 variety by 8%, adopted as Bulgarian standard. On the average, Brio was with smallest grain for the period and ranked 13,3% below standard. The other varieties showed values close to that of Osmanchik 97.

Table 1. 1000 grain mass of rice (paddy), g, 2013-2014

Variety	1000 grain mass - paddy, g					
	2013		2014		Mean	
	g*	%	g*	%	g	%
Osmanchik 97	33,17 bc	100,0	35,52 a	100,0	34,35	100,0
Gala	33,67 b	101,5	32,78 c	92,3	33,23	96,7
Lince	32,52 c	98,0	33,04 bc	93,0	32,78	95,4
Cameo	37,90 a	114,3	36,32 a	108,4	37,11	108.0
Puma	32,96 bc	99,4	33,45 b	94,2	33,21	96,7
Brio	29,31 d	88,4	30,25 d	85,2	29,78	86,7
Mean	33,26		33,56		33,41	

**Data in the same column and heading followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test.*

The hectolitre mass data of the grains are presented in table 2. Considerable differences among varieties by this indicator have been observed. The Puma variety stands out with highest proven values - 48,48 kg, which is 10,5% above standard. The Cameo variety shows lowest hectolitre mass - 42,03 kg. This value is 6,45 kg less than Puma and demonstrates distinction both from Puma and all the other varieties, including the experimental ones. Gala, Lince, Brio and Osmanchik 97 demonstrate close values by this indicator – from 43,25 to 43,90 kg and statistically do not differ among themselves.

The reported results in 2014 were about 1 kg higher on the average. This indicator was influenced by a number of factors including the 1000 grain mass which, although insignificantly, was also higher during the second year. The hectoliter mass was highest with the Puma variety - 49,28 kg thus confirming the tendency in 2013. The hectoliter mass for Brio and Gala was 45,95 and 45,13 kg respectively, while all the others stayed within the range from 43,97 kg (Osmanchik 97) to 44,47 kg (Cameo).

The mean hectoliter mass of the tested varieties for the period was between 43,25 and 48,88 kg. All genotypes performed close to the standard values (± 1,5%), except for the Puma variety (48,88 kg), overweighing Osmanchik 97 by 11,3%.

Table 2. Rice grain hectoliter mass (paddy), kg, 2013-2014

Variety	Hectolitre mass, kg					
	2013		2014		Mean	
	kg*	%	kg*	%	kg	%
Osmanchik97	43,86 b	100,0	43,97 d	100,0	43,92	100,0
Gala	43,90 b	100,1	45,13 bc	102,6	44,52	101,4
Lince	43,79 b	99,8	44,36 cd	100,9	44,08	100,4
Cameo	42,03 c	95,8	44,47 bc	101,1	43,25	98,5
Puma	48,48 a	110,5	49,28 a	112,1	48,88	111,3
Brio	43,25 b	98,6	45,95 b	104,5	44,60	101,5
Mean	44,27		45,53		44,88	

**Data in the same column and heading followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test.*

The data from the biochemical analysis of the rice grain point to the existence of variety dependence for the following indicators: content of crude protein, starch and fats (Table 3). For the period, the protein content in the grain (brown rice) was about 8% on the average. The Turkish variety Gala stood out with highest % of crude protein under the two-year testing conditions (8,89%), followed by the Italian Cameo (8,39%). The tested varieties accumulated about 1% more protein in 2014 which was related to the less favourable conditions for both productiveness formation and the negative correlation between yield and protein content quoted by many authors (Gomez, 1979).

On average, starch content fluctuated in different varieties from 75,17% (Puma) to 78,98% (Osmanchik 97). In accordance with the reported results, the Osmanchik 97 standard variety performed highest and comparatively steady starch content in the two consecutive years.

The tested varieties accumulated comparatively close fat content in the two consecutive years - 3,73% (2013) and 3,57% (2014) on average. The highest reported amount was with the Turkish variety Osmanchik 97 (3,87%) and the Italian Lince variety (3,80%).

Table 3. Biochemical analysis of rice grain (brown), 2013-2014

Variety	Protein %			Starch %			Fats %		
	2013	2014	Mean	2013	2014	Mean	2013	2014	Mean
Osmanchik 97	8,48	8,12	8,30	78,99	78,96	78,98	4,08	3,65	3,87
Gala	8,02	9,58	8,80	78,76	73,10	75,93	3,78	3,62	3,70
Lince	7,72	8,84	8,28	75,77	77,60	76,69	3,72	3,88	3,80
Puma	7,33	8,37	7,85	78,32	72,01	75,17	3,58	3,42	3,50
Cameo	7,67	9,11	8,39	78,07	73,44	75,76	3,75	3,43	3,59
Brio	7,50	8,37	7,94	75,77	77,62	76,70	3,44	3,39	3,42
Mean	7,79	8,73	8,26	77,61	75,46	76,54	3,73	3,57	3,65

Conclusions

The tested Italian (Lince, Cameo and Puma) and Turkish (Osmanchik 97 and Gala) rice varieties have accumulated 1000 grain mass above 32g. It is the Cameo variety (37,11 g/1000 grains) that stands out for the period by 8% above standard. The Brio variety demonstrates smallest grain-size - 29,78 g/1000 grains on average.

The mean grain hectoliter mass of the tested varieties for the period is 44,88 kg. The highest proven value for this indicator (11% above standard) belongs to Puma - 48,88 kg. All the other genotypes perform close values (±1,5% compared to the standard).

The Turkish variety Gala shows highest protein content – 8,80% toward brown rice dry substance. The other varieties perform protein content from 7,85 to 8,30%. The mean starch content for all tested variants is 76,54%, whereas Osmanchik 97 is the only considerably outlined variety for its starch content - 78,98%. The accumulated fats in the grain are from 3,42 (Brio) to 3,87% (Osmanchik 97).

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RESEARCH THE SUITABILITY OF THE LAND FOR GROWTH OF BERRIES AND MEDICAL PLANTS: RECOMMENDABLE FERTILIZATION RATES

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Abstract

The normal growth of most crops ensures in an average annual rainfall 600-800mm. The average annual rainfall in the studied area was 830 mm (with a confidence interval 804-856 mm), and was sufficient to provide the necessary moisture for vegetation for the crops with deep root system. For other crops was necessary to provide irrigation. The terrain is located in the area of Elena town and was in erosive danger. Crop irrigation by gravity way can lead to the intensity of the erosion process and disinterment of the topsoil humus-elluvial layer of the rows. The established values of the indicator pH in the studied plots were significant restriction for the growth of the proposed crops for cultivation. For creation of better conditions for crops growth needs liming on the plots with identified soil acidity. The exceedances of the established concentrations of the easily-mobile exchangeable aluminium and hydrogen, toward conventional limit for toxicity were minor and determine low level of acid toxicity of the soil for the plants. The average content of exchangeable manganese, found in the subsoil horizon exceeds the registered content for the cultivated layer. There is not a tendency of biological accumulation of manganese in the topsoil horizons.

Key words: *soil, fertilization rates, berries, medical plants*

Introduction

Bulgaria has a relatively rich soil and climatic resources, which allow the growth of different plant species. For the properly solve of a number of production problems associated with the effective use of agricultural lands is necessary to know the physiographic features of the area, where are situated soil and climate and in many cases microclimates conditions of the plots, determining the different growth and specific qualities of the growing crop.

Dealing with indicators such as average monthly temperatures during the vegetation period, average dates of sustainable establishment of average day air temperatures above or below a certain limit, temperature amounts for the period with average air temperatures over a certain limit, duration of these periods and of the period without frost, indicators for characterization the conditions of moisture would contribute for the proper and timely implementation of various agronomic practices, ensuring production efficiency (Leeuwen et al., 2004).

The aim of this study was to make the soil and climatic characteristics of the land in the area of Elena town and to assess their suitability for creation and growth of berries and medicinal plants.

Materials and methods

The object of the study is located in the area of Elena town, Elena municipality, Veliko Tarnovo region. The size of the investigated area was 20 ha. In the course of this study was accepted a model to take soil samples, where each sample was taken from the terrain with a soil probe, as sample points are located within the borders of the terrain in a square grid (BDS ISO 10381-2:2002). In two depths 0-25 and 25-50 cm were taken 26 samples points in 4 repetitions. After standard preparation, soil samples were analyzed to establish the indicators: soil texture with fotosedimentograf (Trendafilov and Popova, 2007), Hydrological

characteristics of the soil (Trendafilov and Popova, 2007), Bulk density of the soil by paraffin method, Relative density - pycnometrically, pH - potentiometric in KCl (Arinushkina, 1970); easily mobile exchangeable Al^{3+} and H^{+} titrimetrically by Sokolov (1939); easily mobile exchangeable Mn^{2+} in extract with 1m KCl as the preparation of the extract is performed by the laboratory system for liming by (Palaveev and Totev 1970), (LSVPT-64), and the determination of Mn^{2+} in the extract by AAS (BDS11047, 1995); easily mobile exchangeable Ca^{2+} and Mg^{2+} complexometric by method of Mazaeva, Neugodova and Hovanskaya (Palaveev and Totev, 1970), humus content by Turin method (Trendafilov and Popova, 2007), water-soluble salts (BDS ISO 11265:2002), content of easily absorbable iron, total nitrogen in the soil (BDS ISO 11261: 2002), mobile forms of phosphorus and potassium (GOST 26209-91/01.07.93). Based on the obtained results from the preliminary study was determined harmful acidity and the degree of saturation of soil bases and is proposed plan for melioration of the problem areas and recommended fertilization rates.

Results and discussion

Climatic characteristics

In climatic attitude the studied objects belongs to European continental climatic region, humid continental climatic sub region and before mountainous climatic region. The average altitude of the area is 331m. The average annual rainfall is 830 mm. The rainfall amount during the month with minimum rainfall is 28,75 mm and maximum 125,29 mm. The average annual air temperature is 10,48°C and vary from 9,14 to 11,83 ^{o}C. The winter is cold and summer is cool. For characterization of the climate elements for the studied terrain in the land of Elena town are used data from 10 years period of observation, registered at the nearest meteorological station Elena (Subev, 1959). The average monthly temperature in January vary from -2,88°C to 0,16°C and in July from 19,7°C to 20,6°C (Kichukova, 1983). The average of the absolute minimum air temperature in December is -12,6°C, January -15,7°C and February -13,8°C.

The average temperature amount of biologically active temperatures was 3183°C with a confidence interval from 3066 to 3301°C. Figure 1 shown the probability for recruitment of temperature amounts in the region.

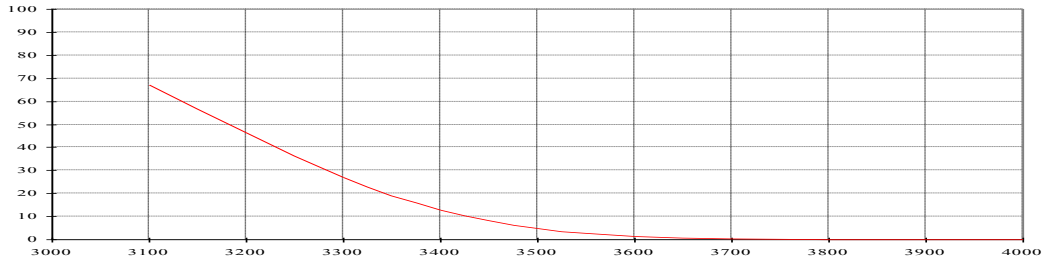

Figure 1. Probability for recruitment (%) of temperature amounts

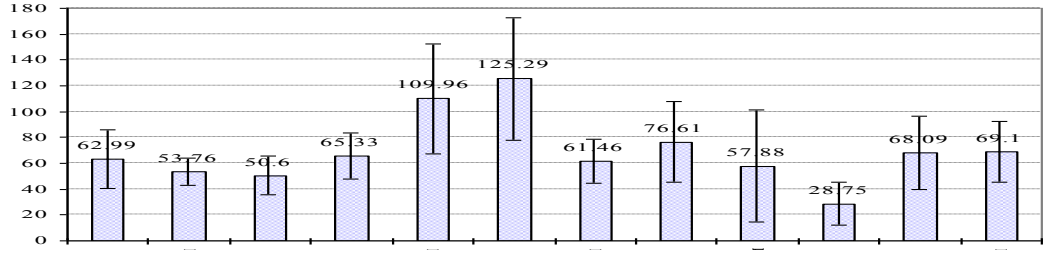

Figure 2. Average monthly rainfall (mm) amounts with intervals of variation (from January to December)

Figure 1 shown, that the probability for recruitment of temperature amount of 3100°C was 67%. The probability for recruitment of temperature amount of 3500°C was 5% and the probability for recruitment of temperature amount higher than 3700°C was 0%. The temperature amount with 75% probability, on the basis of which should make economic calculations about the suitability of the area for growth of berries and medicinal plants in different directions was 3060 °C.

Figure 2 shown the average monthly rainfall amounts with the intervals of their variation.

The normal growth of most crops ensures in an average annual rainfall 600-800mm. The average annual rainfall in the studied area was 830 mm (with a confidence interval from 804 to 856 mm) and was sufficient to provide the necessary moisture for the vegetation for the crops with deep root system, but for the rest crops is necessary to provide irrigation. There is an average annual deficit from -56 to -4 mm. Rainfall amount during the vegetation period (April 1 to September 30) was average 496,53 mm, as the established need was 400-500mm. The average hydrothermal coefficient for April, May, June, July, August September and October shown in Figure 3 (Selyaninov, 1958).

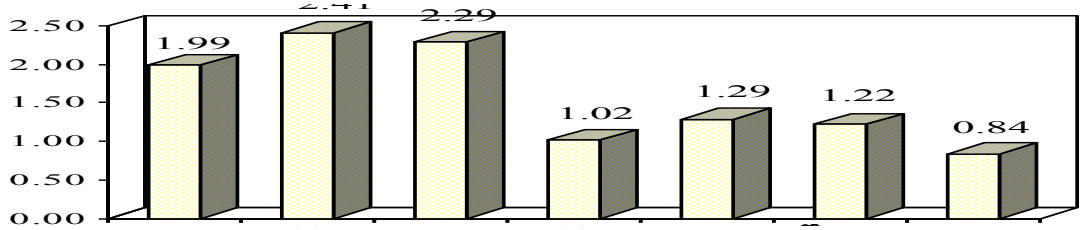

Figure 3. Average values of hydrothermal coefficient by Months (April to October)

General characteristics of the soil

The main soil difference distributed within the studied objects is Haplic luvisols. The soil is formed on sandstones, under the influence of pseudopodzolic process of soil formation. Big role for the formation of Haplic luvisols has the top soil waterlogged. During the wet season iron and manganese compounds are reduced and move into the lower horizons, where in dry conditions formed spots and nodules.

Morphological characteristics

The soils in the studied objects are deep, as the depth of the soil profile is more than 75 cm. Humus-elluvial horizon in not eroded and slightly eroded areas is with depth 20 and over 20 cm. In heavily eroded terrain (Plot 7) humus-elluvial horizon is strongly reduced or absent altogether. In the most heavily eroded sections illuvial horizon is on the top and as a result of which on the terrain is observed red spots.

Morphological description of the soil profile:

A_1 0-25cm. light gray with a yellowish tint, fresh, loose, sandy clay loam, single grained structure, contains iron and manganese nodules, not effervesce from HCl, sharp transition.

B_1 25-50cm. gray-brown, with rust and gray spots, fresh, dense, sandy clay, contains iron and manganese concretions, not effervesce from HCl, gradual transition.

B_2 50-75cm. dark brown, with rust and gray spots, dense, sandy clay, contains manganese and iron concretions, not effervesce from HCl, gradual transition.

The terrain is under erosive danger. Crop irrigation by gravity way can lead to the intensity of the erosion process and to disinterment of the topsoil humus-elluvial horizon of the rows.

Soil texture and physical properties of the soil

The average content of physical clay in the topsoil horizon determined the soil as sandy clay loam. In 25-50cm layer the soil is clay. In both studied depths prevailing the fraction of particles <0,001 mm as the relative part in depth 25-50cm is higher (Table 1). The differentiation of the soil profile is strongly expressed. The average value of the texture coefficient is 2,31. The illuvial horizon is dense, which is a limiting factor in the growth of

shrub crops (raspberry, blackcurrant, blackberry, blueberry). In eventually growth of shrub plants is necessary to perform proper tillage of the soil. It is appropriate periodic soil tillage to 40cm depth or perform of deep melioration loosening. The soil is tilled during the dry period of the year, which provides maximum effect.

Table 1. Average values of the physical indicators of the soil by depths

Indicators	Depth (cm)		
	0-25	25-50	Average
Particles <0,001mm(%)	19,39	44,61	32,00
Physical clay (%)	28,80	61,30	45,05
Texture coefficient	2,31		2,31
Saturation (%)	21,85	20,21	21,03
Porosity %	46,60	45,83	46,21
Bulk density at saturation	1,44	1,46	1,45
Specific density g/cm3	2,7	2,7	2,7

The average values of bulk density did not differ significantly in both studied depths. The data shown a substantial dense of the cultivated soil layer, which should be taken into account in the growth of the proposed crops. The average values of the specific density shown high content of the mineral components in the soil and respectively low organic matter content. The established average specific density did not differ in both studied depths of the soil profile. The total porosity of the soil was low in both, the cultivated soil layer and the subsoil. The average porosity for both studied depths was respectively 46,60 and 45,83%.

Humus content

The average content of organic matter in the soil for all studied plots was 1,00% with a confidence interval from 0,92 to 1,08%. The organic matter slowly decrease in the depth of the soil profile (Figure 4). The established humus content for the cultivated layer did not exceed 1,33%. The humus reserve of the topsoil horizon assessed as low. The average value for all studied objects was 20,9 t/ha and vary in confidence interval from 12,6 to 29,2 t/ha.

The organic matter content in the studied objects can not satisfy the requirements of the crops. The apply of manure in the soil will increase the organic matter content and also is expected to improve the total physical properties of the soil. The rate for apply of manure should be no lower than 30t/ha.

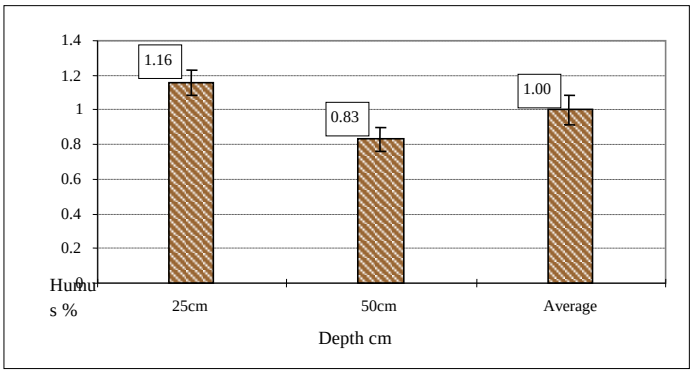

Figure 4. Average humus content in the soil profile depth

Content of water-soluble salts

The content of water-soluble salts in the soil profile depth average for all studied plots was low and did not constitute a restriction for the growth of the selected crops.

Soil pH

The average pH values for the cultivated layer ranged from 3,80 to 5,35. They were higher in the plots, where lime was applied (Plots 1,2,3 and 4). The soil reaction of the cultivated layer

is defined as very strongly acidic to weakly acidic. The pH of the subsoil horizon had lower values compared to the topsoil layer and were in the range of 3,60 to 4,50. The soil reaction of the subsoil horizon was very strongly and strongly acidic (Table 2).

Table 2. Average pH values by depths and plots

pH в KCl	**Depth (cm)**		
Plot	**0-25**	**25-50**	**Average**
Plot 8	4,55	4,50	4,53
Plot 5	5,30	4,50	4,90
Plot 6	5,35	4,10	4,73
Plot 7	4,60	4,00	4,30
Plot 3	4,50	3,95	4,23
Plot 4	3,90	3,80	3,85
Plot 9	3,87	3,83	3,85
Plot 1	3,80	3,60	3,70
Plot 10	4,90	3,80	4,35
Average	4,43	4,06	4,24

The established values of the indicator pH in the studied plots were significant limitation for the growth of the proposed crops. To create better conditions for the growth of the crops on the plots with established harmful soil acidity need liming. On the plots № 1, 4 and 5 is appropriate the growth of blueberries, which grow well in soil solution pH 4,3 to 4,8.

Content of easily mobile exchangeable hydrogen and aluminum

The average content of easily mobile exchangeable Al^{3+} and H^{+} by plots and depths shown in Figure 5.

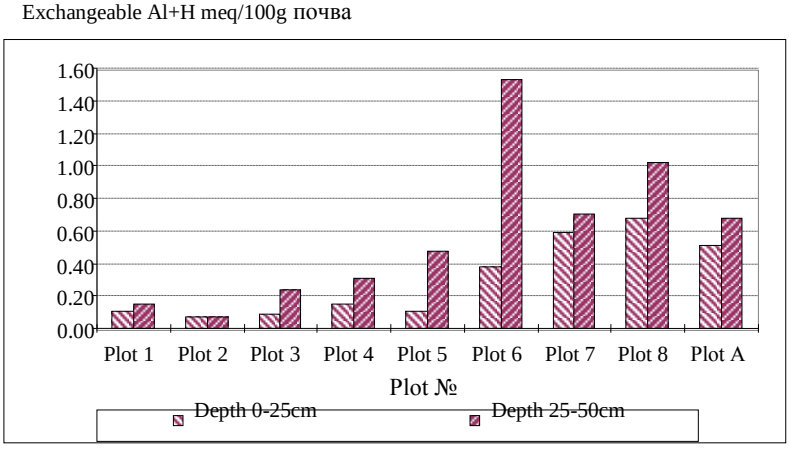

Figure 5. Average content of exchangeable Al + H by plots and depths

The contents of easily mobile exchangeable ions of Al^{3+} and H^{+} increased in the depth of the soil profile, as the excess was most significant in plot №6. The average values of the exchangeable Al^{3+} and H^{+}, found for the depth 25-50cm in plot №6 exceed the registered for the topsoil layer with 4,09 times. This is because of significantly higher sorption capacity of the clay illuvial-metamorphic B horizons. The content of easily mobile exchangeable Al^{3+} and H^{+} for the whole studied soil layer (0-50cm) in the plots was from 0,068 to 0,952 meq/100g soil. The values were lowest in plot №2, where was performed liming. The average value of Al^{3+} and H^{+} for the cultivated layer, found for all studied objects was 0,313 meq/100g soil with a confidence interval from 0,110 to 0,378 meq/100g. The amount of hydrogen + aluminium for the subsoil horizon was average 0,529 meq/100g (confidence interval 0,208 - 0,714 meq/100g), and for the whole analyzed soil layer (0-50cm) - respectively 0,421 meq/100g with a confidence interval from 0,206 to 0,499 meq/100g. The exceedances of the established concentrations of the easily mobile exchangeable aluminium and hydrogen,

toward conventional accepted limit for toxicity were minor and determine low degree of acid soil toxicity for the plants (Figure 5).

Content of easily mobile exchangeable manganese

The average content of exchangeable manganese, found in the subsoil horizon exceeds the registered content for the cultivated layer (Table 3). There is not tendency of biological accumulation of manganese in the topsoil horizons. The data shown, that in the topsoil horizon were created relatively good conditions of aeration and bigger part of manganese is segregated in the form of concretions. In regard to the sensitive crops, the indicated ratio is main criteria for the presence of manganese phytotoxicity of acidic soils. Values greater than the limit numbers according to BDS 17.4.4.07-1997 were found in all studied plots (Table 4). Those plots needs liming to prevent the risk of manganese intoxication.

Table 3. Average content of easily mobile exchangeable Mn by plots and depths

Mn meq/100g	**Depth (cm)**		
Plot	**0-25**	**25-50**	**Average**
Plot 8	0,094	0,100	0,097
Plot 5	0,055	0,097	0,076
Plot 6	0,055	0,120	0,088
Plot 7	0,092	0,128	0,110
Plot 3	0,092	0,134	0,113
Plot 4	0,129	0,169	0,149
Plot 9	0,135	0,145	0,140
Plot 1	0,140	0,167	0,153
Plot 10	0,085	0,143	0,114
Average	0,103	0,130	0,116

Table 4. % ratio between easily mobile exchangeable bases and easily mobile exchangeable manganese

Mn % to Ca и Mg	**Depth (cm)**		
Plot	**0-25**	**25-50**	**Average**
Plot 8	0,92	0,75	0,84
Plot 5	0,48	1,20	0,84
Plot 6	0,54	1,07	0,80
Plot 7	0,93	1,57	1,25
Plot 3	0,85	0,79	0,82
Plot 4	1,58	0,90	1,24
Plot 9	1,81	1,34	1,57
Plot 1	1,42	1,12	1,27
Plot 10	0,60	1,19	0,90
Average	1,12	1,08	1,10

Content of easily mobile exchangeable calcium and magnesium

The content of easily mobile calcium and magnesium in most plots was higher in the subsoil horizon compared to the topsoil (Table 5). This distribution is logical because the soil layer 25-50cm is saturated to a greater degree with clay soil colloids.

Table 5. Average content of easily mobile exchangeable Ca+Mg by plots and depths

Ca+Mg meq/100g	**Depth (cm)**		
Plot	**0-25**	**25-50**	**Average**
Plot 8	11,06	13,94	12,50
Plot 5	11,94	8,23	10,08
Plot 6	10,65	11,40	11,02
Plot 7	10,06	8,23	9,14
Plot 3	11,05	17,27	14,16
Plot 4	8,27	19,06	13,67
Plot 9	7,83	11,30	9,56
Plot 1	9,96	15,04	12,50
Plot 10	14,43	12,10	13,27
Average	10,23	12,85	11,54

Degree of soil saturation with bases and rates for liming

Depends on the obtained values of the indicator V3% was determined the need of liming at plots №6 and 7. For the rest plots the lime rates were defined by the necessary to prevent soil manganese toxicity. The balance lime rates were given in kilograms CaO per hectare. When apply lime meliorants they should be recalculated based on the content of calcium oxide in the meliorant. The rates for some of the most often applied meliorants for chemical melioration of acid soils shown in Table 6. After the last passage of the lime spread aggregate on the field performed shallow tilling - disking, cultivation or milling (for smaller areas). After that the soil was ploughed at a depth of 20-25 cm.

Table 6. Rates for application of lime meliorants in the soil

Plot	CaO kg/ha	Hydrated lime kg/ha	Ground limestone kg/ha	Ash kg/ha
Plot 8	1190	2160	2380	2970
Plot 5	250	460	510	640
Plot 6	440	800	870	1090
Plot 7	590	1080	1190	1480
Plot 3	700	1280	1410	1760
Plot 4	1920	3500	3840	4800
Plot 9	4120	7490	8230	10290
Plot 1	1750	3190	3500	4380
Plot 10	1240	2260	2480	3100
Average	12210	22210	24410	30510

Content of nutrient macro elements

The soil reserve with assimilable forms of nitrogen and potassium were low. The content of assimilable phosphorus was assessed as an average (Table 7). The necessary nutrient macro elements in organic agriculture are applied into the soil, by the use of appropriate fertilizers. Nitrogen is provided by application of manure and potassium by the application of plant ash. By the use of precipitate fertilizer is carried out the liming of areas and simultaneously phosphorus application in the soil.

Table 7. Content of nutrient macro elements

NH_4 mg/kg soil	NO_3 mg/kg soil	Total nitrogen mg/kg soil	P_2O_5 mg/100g soil	K_2O mg/100g soil
28,90	7,88	36,78	14,00	6,00

Conclusions

The soil-climatic conditions of the studied area were suitable for growth of berries and medicinal plants and based on the soil indicators are recommended the following fertilization rates.

Fertilization of raspberry - The main fertilization is in autumn before planting. Application: 50 t/ha manure; 230 kg/ha P_2O_5 or 1150 kg/ha precipitate fertilizer, 180 kg/ha K_2O. The rate of potassium fertilizer in nature is 1800 kg/ha, when used plant ash from coniferous species. Fertilizers must ploughed into the soil to a depth of 25 cm. Those fertilization rates must applied periodically, after establishment of the content of nutrient macro elements in the soil.

Fertilization of blackberry - The main fertilization is with 50 t/ha manure, 290 kg/ha P_2O_5 and 230 kg/ha K_2O. The rates in nature of phosphorus and potassium fertilizer are respectively 1250 kg/ha precipitate fertilizer and 1800 kg/ha plant ash. The application of the fertilizers must be the same way as on the raspberry.

Fertilization of blackcurrant - The main fertilization is with 50 t/ha manure, 270 kg/ha P_2O_5 and 160 kg/ha K_2O. The rates in nature of phosphorus and potassium fertilizer are

respectively 1350 kg/ha precipitate fertilizer and 1800 kg/ha plant ash. The blackcurrant is sensitive to soil acidity. In order to create suitable conditions for growth, on the areas identified for its growth is necessary to control the soil acidity. When found necessary must apply once more liming.

Fertilization of blueberry - The main fertilization is with 40 t/ha manure, 190 kg/ha P_2O_5 and 140 kg/ha K_2O. The rates in nature of phosphorus and potassium fertilizer are respectively 1120 kg/ha precipitate fertilizer and 1400 kg/ha plant ash.

Fertilization of strawberries - Before planting of the strawberries, must liming to neutralize harmful soil acidity. The fertilization is with 520 kg/ha P_2O_5 applied as precipitate fertilizer and with 2000 kg/ha wood ash of coniferous species. The rate of nitrogen is covered by the application of manure.

Fertilization of Melissa officinalis - The main fertilization is with 40 t/ha manure, 260 kg/ha P_2O_5 and 180 kg/ha K_2O. The rates in nature of phosphorus and potassium fertilizer are respectively 1300 kg/ha precipitate fertilizer and 1800 kg/ha plant ash.

Fertilization of chamomile - With lime application on the areas, for growth of chamomile, must apply 30 t/ha manure. If the previous crop before the chamomile is fertilized with manure, must not apply fertilizer.

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POLYMORPHISM OF HIGH MOLECULAR WEIGHT GLUTENINS IN WHEAT ADVANCED LINES ORIGINATED FROM CROSSES WITH D-GENOME DERIVED SYNTHETICS

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Abstract

The objective of this study was the allelic variation at the *Glu-1* locus of selected advanced lines from ten cross-combinations, obtained by crossing synthetic hexaploids (2n=42, $BBA^uA^uD^tD^t$) with bread wheat varieties. In total, 52 genotypes in BC_1F_4 and BC_3 were investigated by SDS-PAGE electrophoresis and grouped in 20 HMW-GS combinations at the three loci of group 1 chromosomes (*Glu-A1*, *Glu-B1* and *Glu-D1)*. At the *Glu-D1* locus, the genetic diversity was relatively high-H=0.63, as calculated by Nei index. Maximum concentration of hereditary material in favorable quality subunits 1Dx5+1Dy10 and 1Dx1.5+1Dy10 was observed. Subunit pair 1Dx1.5 + 1Dy10, coding by *ah* allele, was different from the *T. aestivum* genes. This allele was inherited from *Aegilops tauschii* (2n=14, D^tD^t) through the synthetic wheat and might have a significant effect on the bread-making quality in bread wheat. The average score of the high molecular glutenin combinations was calculated as 6.50. According to its share in the formation of this value, the investigated loci ranked as followed: *Glu-B1>Glu-A1>Glu-D1*. Twelve biotypes showed subunit 1Ax1.1, that is uncommon for the bread wheat, representing a reservoir of expanding allelic diversity for HMW-GS along with the D^t-genome derived genes.

Key words: synthetic hexaploid wheats, *Aegilops tauschii*, *T. aestivum*, SDS-PAGE, HMW-GS

Introduction

In the last century, modern wheat breeding caused a loss of beneficial genes in the newly created *T. aestivum* varieties. Contrasting to this, local landraces and wild wheats possessed a diversity of alleles, which are important for resistance to biotic and abiotic stresses and grain quality in wheat. Many synthetic amphiploids and nearly isogenic lines have been developed as bridges to transfer valuable traits in common wheat (Jauhar and Peterson, 2006; Plamenov and Spetsov, 2011; Hu et al., 2013).

Aegilops tauschii (2n=14, DD), the donor of D-genome in wheat (*T. aestivum*, 2n=42, BBA^uA^uDD) has many breeding traits (Gill et al., 1996; Tang et al., 2008; Trethowan and Mujeeb-Kazi, 2008), including seed proteins of significant importance for the end-use quality in wheat (Pena et al., 1995; Tang et al., 2008). *Ae. tauschii* accessions have been crossed to tetraploid *Triticum* species to obtain synthetic hexaploid wheats or directly to bread wheat varieties. The D-genome derived synthetics beard various genes in the Glu-D1 locus for grain quality, which formed interesting combinations with alleles in Glu-A1 and Glu-B1 loci (Peña et al.,1994, 1995; Hsam et al., 2001).

Gluten quality is a target of the modern wheat breeding. It consists of two prolamins - glutenins and gliadins (Atanasova et al., 2009; Rasheed et al., 2012; Hu et al., 2013). Glutenins, including high-molecular-weight and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS), form a long chain of polypeptides, connected in between through disulfide strings to determine exclusively dough viscoelastic properties. HMW-GS subunits are coded by genes at *Glu-A1*, *Glu-B1* and *Glu-D1* and localized in long arms of 1A, 1B and

1D chromosomes. Each single locus is responsible for one x- and y-type subunit. These loci are highly polymorphic in nature without the influence of environment (Payne et al., 1981). The allelic variation at the three loci coding HMW-GS has been used either independently or in combination with other traits to estimate the genetic variation in species, hybrids and populations. The genotypes received Glu-1 скор, which has been used as a criterion for bread making quality (Payne, 1987). Although HMW-GS constitute only 10% of the storage in seed endosperms, about 67% of variation in bread-making quality in *T. aestivum* is due to different allelic variants of glutenins (Payne et al., 1984). Thus, the analysis of HMW-GS is a prerequisite and basic measure in breeding for wheat improvement.

The aim of this study was to characterize the high-molecular-weight glutenin subunits in selected advanced plants from hybrid generations of ten cross-combinations between two synthetic D-genome derived lines (*T. dicoccum*/*Ae. tauschii*) and *T. aestivum* varieties. Establishing the glutenin pattern in single plants at early stage of breeding will be very important for the fast creation of homozygous lines and their immediate involvement in breeding process.

Materials and methods

Materials: Plants in BC_1F_4 and BC_3 generations selected from ten cross-combinations, were included in the investigation (Table 1). First, 5 hybrids between three bread wheat cultivars and two synthetic wheats (*Triticum dicoccum*/*Aegilops tauschii*) were produced. The hybrids were crossed to eight *T. aestivum* varieties to develop 10 cross-combinations which were advanced on the field by selecting different plants on spike colour and shape. Synthetics (AD530 and 532) involved hybrid parent as tetraploid component in crosses to *Ae. tauschii* accessions (Table 2).

Table 1. Pedigree of the cross-combinations obtained by crossing bread wheat cultivars with two synthetic D-derived amphiploids and resulted HMW-GS patterns

Breeding no.	P e d i g r e e	Generation of seed	Checked plants/no	Total grains analyzed/no	HMW-GS patterns
128	Korona/$72F_1$/Aglika	BC_3F_2	5	52	3
136	Kristora/$74F_1$/Aglika	BC_3F_2	6	52	2
166	$72F_1$/Todora/Aglika	BC_3F_2	5	45	3
167a	$74F_1$/Aglika/Goritsa	BC_3F_2	6	48	1
168	$74F_1$/Enola/Kristora	BC_3F_2	5	32	3
168a	$74F_1$/Enola/Enola	BC_3F_2	5	35	2
177	$90F_1$/Perla-2/Enola	BC_3F_2	6	32	1
181	$98F_1$/Alana/Kristora	BC_3F_2	5	32	1
118	Enola/$74F_1$	BC_1F_5	5	40	1
110	Aglika/$75F_1$	BC_1F_5	4	38	3
		Total	52	406	20

$72F_1$(Polena/AD530); $74F_1$(Slavea/AD530); $75F_1$(Slavea/AD532); $90F_1$(AD530/Aglika); ` $98F_1$(AD532/Aglika).

Table 2. Origin of the two synthetic lines used in crosses to eight *T. aestivum* varieties

Breeding no	C r o s s[1]	Genome formula (2n)[1]
530	45390/45398/[2] *Ae. tauschii* ac.19088	$BBA^uA^uD^tD^t$
532	45390/45398)/ *Ae. tauschii* ac. 22744	$BBA^uA^uD^tD^t$

[1],Genome formule are according to Goncharov et al. (2009);
[2],*Triticum dicoccum* accessions.

Methods: HMW glutenins were extracted according to Singh et al. (1991). The electrophoresis run on vertical apparatus as classical one-dimensional 12% polyacrylamide gel SDS – PAGE (Laemmli, 1970). Arrangement and numbering of HMW-GS in Glu-A1 and Glu-B1 loci in wheat was carried out (Payne and Lawrence, 1983). Alleles in Glu-D1 locus were described according to William et al. (1993). Quality score was calculated (Payne et al.,1980) by summing individual scores of separate high-molecular-weight glutenin subunits. Genetic diversity in a locus was determined by the Nei index (Nei, 1973) by means of: $H = 1-\sum Pi^2$, where *H* and *Pi* designate the index of diversity and frequency of alleles in a locus, respectively. Allelic frequencies were calculated as a sum total of accessions divided by accession number.

Results and discussion

Generally, 406 seeds were investigated, between five and ten grains per selected plant (Table 1). Six cross-combinations generated two and three HMW-GS patterns. Subunit 2* was the most frequent glutenin in Glu-A1 and the index for genetic diversity was comparatively high, H = 0.71. Subunit N was found very rarely (9.6%). Subunits 2* and 1, which displayed high frequency, contributed to bread making quality in wheat. Genotypes with subunit 1.1, originating from the synthetic wheat, should be valuable for wheat improvement, because the allele *x*, coding this glutenin unit, appeared as a new gene for the bread wheat genome. The score of Glu-A1 was estimated as 2.11 without the effect of allele *x*, which score is not calculated yet (Табл. 3).

Three alleles (*b, c, f*) were found in Glu-B1 coded pairs 7+8, 7+9 and 13+16, respectively. Its genetic diversity is H=0.51. The subunit 7+9 had the biggest part 65.4%, the next were 13+16 (23.1%) and 7+8 (11.5%) pairs, which positively influenced the bread quality. The quality score in this locus was 2.35, compared to the maximum value of three (Табл. 3).

In locus Glu-D1, the genetic diversity (H=0.63) was higher than in locus Glu-B1. Three alleles (*d, a* and *ah*) were again determined in this locus, providing 5+10, 2+12 and 1.5+10 pairs, respectively. Allele *ah*, coding 1.5+10 subunits, is a new gene in the glutenin spectrum of 21 advanced *T. aestivum* genotypes. It originated from the diploid *Ae. tauschii* through the synthetic amphiploid, used in crosses to bread wheat varieties. The glutenin-score was calculated as 2.04 from the maximum value of four. The subunits 1.5+10 have no score estimate at present, so it is expected this value to be increased. Some information is available for the positive effect of allele *ah* on bread-making qualities (Peña et al., 1995; Tang et al., 2008). It was proved, that biotypes with 1.5+10 pair in combination with 7+8 in Glu-B1 and subunit 1 in Glu-A1 were better for grain quality than the combination of 5+10 with the same alleles of the two loci (Peña et al., 1995; Tang et al., 2008). Li et al. (2009) stated that 5+10 were the best valuable subunit pair for quality among all others in Glu-D1 locus, while Vaiciulyte-Funk et al. (2015) found 2+12 in combination with Null, 6+8 and HMW-glutenin quantity of 10.3-11.4% being most suitable for ring shaped bread production.

Table 3. Frequency and genetic diversity in HMW-GS among the advanced lines

Locus	Allel	Subunit	Glu-1 score	Selected plants/no	Frequency, %
Glu-A1	c	N	1	5	9.6
H=0.71	a	1	3	17	32.7
Score>2.11 (3.00)	x	1.1	-	12	23.1
	b	2*	3	18	34.6
Glu-B1	b	7+8	3	6	11.5
H=0.51	c	7+9	2	34	65.4

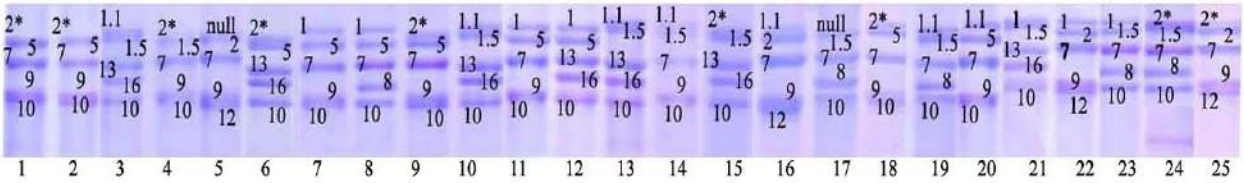
Score-2.35 (3.00)	f	13+16	3	12	23.1
Glu-D1	d	5+10	4	22	42.3
H=0.63	a	2+12	2	9	17.3
Score>2.04 (4.00)	ah	1.5+10	-	21	40.4
Glu-score 6.50 (10.00)					

A final result of all recombination among the subunits in the three loci were twenty HMW-GS patterns (Table 4, Fig. 1). The Glu-1 score varied from 5 до 10, with a medium of 6.50. This value does not include subunits 1.1 (in 12 progenies) and 1.5t+10t, which have no score values at present. According to the share in forming the glu-score, the loci were arranged as following: Glu-B1>Glu-A1>Glu-D1.

The resulted HMW-GS configurations contributed differently to grain quality. Three of them, 2*, 7+9, 5+10; 1, 7+9, 5+10 and 1.1, 7+9, 1.5+10 monitored the basic part (36.5 %) of glutenins. They were connected with high (score 9) and comparatively good (score 7) quality. About 23% of the glutenin potential was under control of four spectrums with frequency of 5.7%. The glu-score of two glutenin compositions was 7 and assigned to good grain quality. The rest 13 HMW-GS patterns, each was found once or twice in the selected lines, controlled 40.2% of glutenins in the plants investigated. We suppose, progenies with 1.5t+10t pair in Glu-D1 and a subunit 1.1 in Glu-A1, should produce grain of good to high quality (Table 4).

Table 4. HMW-GS patterns of selected lines from ten cross-combinations in BC_1F_5 and BC_3F_2 generations

HMW-pattern	Allele	Glu-1 score	Progeny/ no	Frequency, %
N, 7+9, 2+12	c, c, a	5	2	3.9
N, 7+9, 1.5t+10t	c, c, ah	-	2	3.9
N, 7+8, 1.5t+10t	c, b, ah	-	1	1.9
1.1, 7+9, 2+12	x, c, a	-	1	1.9
1.1, 7+9, 5+10	x, c, d	-	2	3.9
1.1, 7+9, 1.5t+10t	x, c, ah	-	5	9.6
1.1, 7+8, 1.5t+10t	a, b, ah	-	1	1.9
1.1, 13+16, 1.5t+10t	a, f, ah	-	3	5.8
1, 7+9, 2+12	a, c, a	7	3	5.8
1, 7+9, 5+10	a, c, d	9	6	11.4
1, 7+8, 5+10	a, b, d	10	2	3.9
1, 7+8, 1.5t+10t	a, b, ah	-	1	1.9
1, 13+16, 5+10	a, f, d	10	2	3.9
1, 13+16, 1.5t+10t	a, f, ah	-	3	5.7
2*, 7+9, 2+12	b, c, a	7	3	5.7
2*, 7+9, 5+10	b, c, d	7	8	15.3
2*, 7+9, 1.5t+10t	b, c, ah	-	2	3.9
2*, 7+8, 1.5t+10t	b, b, ah	-	1	1.9
2*, 13+16, 5+10	b, f, d	10	2	3.9
2*, 13+16, 1.5t+10t	b, f, ah	-	2	3.9
			Total 52	100.0



Фиг. 1. HMW-GS composition in advanced wheat lines originated from ten cross-combination with participation of two synthetic D-genome derived wheats (12% SDS-PAGE):
1. Bezostaya 1 (check); 2.128-1; 3. AD530-4 (check); 4.128-2; 5.128-3; 6.136-1; 7.Aglika (check); 8.136-2;9. Polena (check); 10.532-2 (check); 11.166-1; 12.166-2; 13.168-1; 14.118-1; 15.168a-1; 16.181-1; 17.110-1; 18.177-1; 19.110-2; 20.167a-1; 21.168-2; 22.166-3; 23.168a-2; 24.110-3; 25.168-3.

Conclusions

1. Analysis of glutenins in 52 plants selected in BC_1F_4 and BC_3 from ten cross-combinations with participation of two synthetic D-genome derived wheats (*T. dicoccum*/*Ae. tauschii*) classified them in twenty HMW-GS different patterns.
2. Twenty one progenies were found to have the subunits 1.5t+10t, originated from the synthetic wheats. One progeny (seeds in BC_3F_2) displayed the spectrum Glu-A1(1)+Glu-B1(7+8)+Glu-D1(1.5t+10t) which seemed to be a combination for high grain quality.
3. Twelve progenies had the subunit 1.1 in the Glu-A1 locus. Allele *x*, coding this glutenin, was considered as a new gene for the wheat genome. Lines were grouped in 5 HMW-GS patterns and could be also regarded as valuable resources for enlargement the genetic diversity in wheat.

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INVESTIGATION ON THE RESISTANCE OF COMMON WINTER WHEAT LINES TO NATURAL INFECTION OF P. STRIIFORMIS

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Abstract

Stripe rust is an important disease on wheat which can cause significant yield losses and deteriorate grain quality. The ability of the pathogen to mutate and replicate quickly, as well as to overcome vast spaces airborne makes it threatening to the resistance of the new cultivars. This imposes the necessity to constantly work on the development of new improved cultivars with higher durable resistance.

Our investigation presents the results from the study on the resistance of common winter wheat lines under natural infection of *P. striiformis.*

Fifty-nine common winter wheat lines at adult stage were tested for resistance to a natural population of *P. striiformis* at Dobrudzha Agricultural Institute – General Toshevo, Bulgaria during season 2013 – 2014. A certain degree of resistance was shown by 71 % of the lines. In 8.4 % of the lines there was no attack by stripe rust. High resistance was found in 17 % of the investigated material.

The lines which reacted as resistant were 33.9 %, while moderate resistance was found in 11.8 % of the lines. In a large part of the investigated material the resistance to stripe rust was combined with good field resistance to leaf and stem rust and to powdery mildew, and with other valuable properties such as high bio chemical index of grain determined in our previous investigations. The purposeful use of own and foreign sources of resistance in breeding has lead to the development of initial material combining disease resistance with other valuable economic properties.

Keywords: resistance, common wheat lines, *P. striiformis*, natural infection

Introduction

Stripe rust with cause agent *P. striiformis*is a main disease on wheat worldwide which can cause considerable losses of yield and grain quality (Singh et al., 2004; Long, 2007). Some authors point out that stripe rust is becoming an increasingly serious problem in the last 40 years (Line, 2002; Chen, 2005).

The occurrence of stripe rust in Bulgaria is sporadic. It can be found in regions with more humid and cool climate such as the coastal areas and the high fields in the piedmonts. In the recent years, however, it is observed all over the country due to the climatic changes favoring its development and propagation.

Because of the occurrence of new airborne races and the ability of the pathogen to mutate fast, the genes for resistance are being overcome more frequently (Chen, 2005). This imposes the necessity to work constantly for the development of new improved cultivars with more durable resistance. Therefore the strategy is to use closely related and wild species as new sources of resistance. The studies have shown that especially valuable in this respect are some forms from genera *Triticum, Agropyron, Aegilops* (Riley, 1968; Sears, 1961).

The aim of this study was to investigate the response of common winter wheat lines to natural infection of *P. striiformis* with a view of their most rational usage as sources of resistance in breeding.

Materials and Methods

Fifty-nine common winter wheat lines and their resistance stage to natural infection of stripe rust at adult were subjected to investigation. The studies were carried out at Dobrudzha Agricultural Institute – General Toshevo, Bulgaria, under field conditions during harvest season 2013 – 2014. The lines were derived from 12 crosses. The mother components of the presented lines originated from complex crosses of cultural wheat to wild and synthetic forms. Foreign and Bulgarian cultivars and lines carriers of genes which determine resistance to diseases were also involved. The pedigree of the investigated lines is given in Table 1.

Table 1. Breeding number and pedigree of the investigated lines

Line/No	Pedigree
20-297-	(Nadadores 63 x San Pastore x TPR 354) x (Russalka x *Ag. glaucum* x Avrora) x 306/92-71
20-300-	(Dobrudzha 1 x Sadovo 1) x (M.amber x 73/557) x Tompus Blank x Delta
20-303-	(Dobrudzha 1 x Sadovo1) x (M.amber x 73/557) x Tompus Blank x (11- 8 x Trayana)
20-306-	(Sonora 64 x Wisconsin 245) x (Nadadores 63 x San Pastore x TPR 354) x (Russalka x *Ag. glaucum* x Avrora) x (M.amber x 73/ 557) x Sadovo1
20-309-	(Sonora 64 x Wisconsin 245) x (Nadadores 63x San Pastore x TPR354) x (Russalka x *Ag. glaucum* x Avrora) x (Michigan x 73/ 557) x 15-92
20-318-	(Sonora 64 x Wisconsin 245) x (Nadadores 63 x San Pastore x TPR 354) x (Russalka x *Ag. glaucum* x Avrora x Ogosta) x (Charodeika x Vratsa x 268A) x (5 –IV – IP x Tompus Blank) x 1007-3 KK
20-324-	(Nadadores 63 x San Pastore x TPR 354) x (Russalka x *Ag. glaucum* x Avrora x Ogosta) x 5367 ИС x Svilena
20-327-	(Nodadores 63 x San Pastore x TPR 354) x (Russalka x *Ag. glaucum* x Avrora x Ogosta) x 5367 IS x C 92/46-6 NT
20-330-	(Nadadores 63x San Pastore x TPR 354) x (Russalka x *Ag. glaucum* x Avrora x Ogosta) x 5367 IS x B 92/14-1 NT
20-333-	(Nadadores 63 x San Pastore x TPR 354) x (Russallka x *Ag. glaucum* x Avrora x Ogosta) x Slavyanka 196
20-336-	(Sonora 64 x Wisconsin 245) x (Nadadores 63 x San Pastore x TPR 354) x (Russalka x *Ag. glaucum* x Avrora) x Klotest x (Michigan x 73/ 557) x B 91/ 62-7 NT
20-339-	(Erythrospermum 19-16 x Vratsa) F1 x (Sonora 64 x Wisconsin 245) x (334C x Zlatna dolina x 720-28) x (Michurinka x 73/557 x 196 MC) x Niconus

The sowing of the tested lines was done manually, each line being planted in 5 rows 1m long, with 25 cm interspacing. Cultivar Michigan amber was used as a check susceptible to *P. striiformis*. The attacking rate on the check cultivar over the years was 60 %.

The attacking rate of stripe rust was read as percent of infected leaf area according to the modified scale of Cobb (Peterson, 1948).

The level of resistance was calculated by comparing the relative disease severity (DS) of each cultivar to that of the susceptible check M. amber. The relative DS was calculated by the formula as follows:

Average disease severity (DS) of the cultivar / Average disease severity (DS) of M. amber x 100.

The field response of the host plants to stripe rust is presented in Table 2.

Table 2. Field response of host plant to stripe rust

Reaction	Description	Observation	Constant Value
NoDisease	No visible infection	0	0
Resistant	Visible chlorosis or necrosis, No uredia are present	R	0,2
Resistant – Moderately Resistant		R-MR	0,3
Moderately Resistant	Small uredia surrounded by chlorotic/necrotic areas	MR	0,4
Mod. Res. - Mod. Sus.		MR-MS	0,6
Moderately Susceptible	Uredia medium size with no necrotic margins but possibly some distinct chlorosis	MS	0,8
Mod.Sus.- Susceptible		MS-S	0,9
Susceptible	Large uredia without necrosis or chlorosis	S	1

Disease severity and host response data are often combined into a single value called the coefficient of infection (CI) (Table 2). The (CI) is calculated by multiplying the severity times a constant for host response: where immune I=0.0, R=0.2, MR=0.4, MS=0.8, and S=1(Roelfs et al., 1992). The field responses and the constant values are presented in Table 2.
The cultivars were classified into:
Immune – 0; Very resistant (0-5); Resistant (6-25); Moderately Resistant (26-45); Moderately Susceptible (46-65); Susceptible (66-100).

Results and Discussions

The reaction of the investigated common winter wheat lines and their coefficient of infection and rating are presented in Table 3. Among the investigated 59 lines, 71 % demonstrated certain degree of resistance. In 8.4 % of the lines, stripe rust attack was not found. These lines (20-330-2-5, 20-333-1-2, 20-333-2-4, 20-333-4-14 and 20-333-5-5) were registered as immune. Three lines were tested from cross No 20-297- . Line 20-297-1-12 reacted with (CI=3.3), which places it in the group of very resistant lines (VR). Line 20-297-1-1 responded with(CI = 6.6) and also fell in the group of resistant lines (R). The line combines resistance to stripe rust with high resistance to leaf rust and powdery mildew, and demonstrated resistant to moderately resistant response to the cause agent of stem rust (Ivanova et al., 2009). The third line from this cross, 20-297-1-13, reacted as moderately susceptible (MS) with (CI = 53.3).
The pedigree of the lines from this cross involved the combination (Russalka x *Ag. glaucum* x Avrora). It is known that the wheat x couch grass (*Agropyrum repens*) hybrids are characterized with very high multi barrier resistance to stripe rust at adult stage (Malinsky et al., 1984).

Table 3. APR of common winter wheat lines to the cause agent of stripe rust *P. striiformis*

There is data that cultivar Russalka possesses very high multi barrier resistance to stripe rust. Malinsky and Mihova (1985) proved that the resistance in cultivar Russalka to race 41 E136 is controlled by one dominant gene and one recessive gene.

Savov and Donchev (1982) presented researches on the resistance at adult stage of cultivar Russalka to leaf rust and powdery mildew. Cultivars Nadadores 63, TPR 354, Sonora 64 tend to be varieties with durable and horizontal resistance to *Puccinia graminis tritici* and this resistance

Lines	Reaction	CI	Rating	Lines	Reaction	CI	Rating
20-297-1-1	10MR	6.6	R	20-309-5-5	20MR	13.3	R
20-297-1-12	5MR	3.3	VR	20-309-6-3	20MR	13.3	R
20-297-1-13	40MS	53.3	MS	20-309-6-4	20MR	13.3	R
20-300-2-2	10MR	6.6	R	20-318-1-6	20MR	13.3	R
20-300-2-2 awned	5R	1.6	VR	20-318-3-5	20R	6.6	R
20-300-2-6	5R	1.6	VR	20-318-4-16	5R	1.6	VR
20-300-2-18	5R	1.6	VR	20-324-1-4	10MR	6.6	R
20-300-3-5	5R	1.6	VR	20-327-3-5	80S	130.0	VS
20-300-3-16	5MR	3.3	VR	20-327-5-13	10MS	13.3	R
20-303-1-12	60S	100.0	VS	20-330-1-2	5MR	3.3	VR
20-303-1-3	40MR	26.6	MR	20-330-2-5	0	0	I
20-303-2-4	40MS	53.3	MS	20-333-1-2	0	0	I
20-303-2-5	30MS	40.0	MR	20-333-2-4	0	0	I
20-303-2-15	40MS	53.3	MS	20-333-4-14	0	0	I
20-303-4-12	40MR	26.6	MR	20-333-5-5	0	0	I
20-303-4-18	30MR	20.0	R	20-336-1-1	30MS	40.0	MR
20-306-5-2	10MR	6.6	R	20-336-1-2	30S	50.0	MS
20-306-5-3	20MR	13.3	R	20-336-1-14	20MR	13.3	R
20-306-5-5	10MR	6.6	R	20-336-1-23	60S	100.0	VS
20-306-5-6	20MS	26.6	MR	20-336-2-4	30S	50.0	MS
20-306-5-11	20MR	13.3	R	20-336-2-5	40S	66.6	S
20-306-6-2	40MS	53.3	MS	20-336-3-23	10MS	13.3	R
20-306-6-2 awned	10R	3.3	VR	20-336-3-24	10MR	6.6	R
20-309-3-2	20R	6.6	R	20-336-4-2	30MS	40.0	MR
20-309-3-3	40MS	53.3	MS	20-336-4-3	40S	66.6	S
20-309-3-11	20MR	13.3	R	20-336-4-5	80S	130.0	VS
20-309-4-11	20MR	13.3	R	20-336-5-2	20MS	26.6	MR
20-309-4-12	40MS	53.3	MS	20-339-3-11	40S	66.6	S
20-309-4-16	60S	100.0	VS	20-336-6-11	40S	66.6	S
20-309-5-1	5MR	3.3	VR				

has remained invariable for more than 20 years (Karjin, 2002). The involvement of all these cultivars in the cross explains the complex character of resistance in the above lines.

From the cross with breeding number 20-300-, a total of 6 lines were tested. Five of them (20-300-2-2 awned, 20-300-2-6, 20-300-2-18, 20-300-3-5 and 20-300-3-16) responded as very resistant (CI = 1.6) and one (20-300-2-2) as resistant (CI = 6.6) (Table 3). The high resistance of these lines to stripe rust was combined with expressed full resistance to all pathotypes of leaf rust at young stage and high field resistance to leaf and stem rust and powdery mildew (Ivanova et al., 2009; Ivanova et al., 2015). The mother component of the cross involved the combination (M. amber x 73/557).

Line 73/557 is a cross between the amphidiploid (*T. timopheevi var. typica* x *Ae. squarrosa var. strangullata*) and cultivar Avrora. It is possible that the high resistance to stripe rust demonstrated by these two lines came from genes transferred from the wild species or from

cultivar Dobrudzha 1, for which there is data that carries partial resistance to stripe rust (Karjin et al., 1994). The lines from the cross 20-303- are rather diverse with regard to their response to *P. striiformis.* Line 20-303-4-18 belonged to the group of resistant lines (CI=20.0). Three of the lines had moderate resistance (20-303-1-3, 20-303-2-5, 20-303-4-12), two were with moderately susceptible reaction (20-303-2-4 and 20-303-2-15) and one (20-303-1-12) fell in the group of the highly susceptible lines. Cultivar Dobrudzha 1 and line 73/557 were also involved in the mother component of the lines from this cross, but the father component in this case was the combination (11-8 x Trayana), which is in fact the new cultivar Lider recently developed at Dobrudzha Agricultural Institute. In a previous investigation (Ivanova, 2015) it was found that cultivar Lider responded with a resistant reaction to natural infection of *P. striiformis.* Seven lines were tested from the cross 20-306-. Only one of the lines in this cross reacted as moderately susceptible. The rest demonstrated degree of resistance within the range from moderate to high (Table 3). Lines 20-306-5-2, 20-306-5-3, 20-306-5-5 and 20-306-5-11 showed resistant reaction to stripe rust. Line 20-306-5-2 combined resistance to stripe rust with high resistance to leaf rust and powdery mildew (Ivanova, 2015). Lines 20-306-5-3, 20-306-5-5 and 20-306-5-11 combined resistant reaction to stripe rust with high resistance to leaf and stem rust. Line 306-6-2 (awned form) had expressed high resistance to stripe rust also combined with high resistance to the other two types of rust (Ivanova, 2015). The complex character of resistance in these two lines was mainly due to the cultivars with durable resistance included in them, such as Sonora 64, Wisconsin 245, Nadadores 63, San Pastore, TPR 354, the combination (Russalka x *Ag. glaucum* x Avrora) and the line 73/557.

Ten lines were tested from the cross 20-309-. Three of them demonstrated moderate and high susceptibility. The rest reacted as resistant, and one of them, 20-309-5-1, demonstrated high resistance. The high resistance to stripe rust in this line was combined with high resistance to leaf rust and powdery mildew and with high to moderate resistance to stem rust (Ivanova, 2009). Three lines were tested from the cross 20-318-. Two of them reacted as resistant, and one of them demonstrated high resistance. The resistance to stripe rust of line 20-318-1-6 was combined with high resistance to leaf rust and powdery mildew, and with high to moderate resistance to black rust. Line 20-318-3-5 combined resistance to stripe rust with high resistance to powdery mildew and with high to moderate resistance to brown and stem rust (Ivanova, 2015).

Line 20-318-4-16 was with expressed high resistance to stripe rust combined with high resistance to powdery mildew and was very resistant to resistant to leaf and stem rust (Ivanova, 2015). These lines also involved cultivars with durable resistance such as cultivar Vratsa, which carried horizontal resistance to stripe rust (Karjin, 2003).

One line was tested from the cross 20-324-, which also exhibited resistant reaction (CI = 6.6) to the natural population of stripe rust. The resistance to stripe rust in this line was combined with high resistance to brown rust and powdery mildew (Ivanova, 2009). Two lines were tested from the cross 20-327-. One of them reacted as very susceptible (CI = 130.0), while the other demonstrated resistant reaction. Two lines were tested from the cross 20-330-. In one of them no attacking rate was registered, i.e. it behaved as immune, while the other one responded as very resistant (CI = 3.3). Four lines from the cross 20-333- were tested. None of the lines from this cross was attacked by stripe rust, i.e. they demonstrated immune reaction.

The investigations of Mihova showed that the resistance at adult stage was typical of wheat with regard to stripe rust; she pointed out that the Bulgarian cultivars Ogosta, Tundzha and Vratsa were carriers of such resistance (Karjin et al., 1994). The presence of these cultivars in the investigated lines from crosses 20-318, 20-324-, 20-327-, 20-330- and 20-333- was probably the reason for the exhibited resistance to stripe rust.

Thirteen lines were tested from the cross 20-336-. The responses of these lines were rather versatile ranging from resistant to very susceptible (R-VS). Six of the lines demonstrated certain degree of resistance, three of them responding as resistant and three as moderately resistant. The mother component of the lines from the cross 20-339- involved cultivar Erythrospermum 19-16. Mihova found high level of horizontal resistance to stripe rust in this cultivar (Karjin et al., 1994). We, however, tested only one line from this cross and it showed susceptible reaction.

Conclusion

Among the tested 59 common winter wheat lines at adult stage for resistance to the natural population of *P. striiformis*, 71 % demonstrated certain degree of resistance. 8.4 % were registered as immune. The resistance to stripe rust in the greater part of these lines was combined with resistance to the other economically important diseases on wheat, as well as with other invaluable properties such as high bio chemical index determined in our previous researches. The high and complex resistance makes these lines especially valuable for breeding. They are important initial material containing original resistance which has been efficient for more than 30 years now.

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NEW COMMON WINTER WHEAT LINES WITH RESISTANCE TO LEAF RUST (P. TRITICINA)

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Abstract

Wheat is the most important cereal crop in global agricultural economy and cultivated in diverse agroclimatic regions of the world. Breeding for disease resistance is the most economically and environmentally safe method to reduce crop losses. The long term success of breeding for disease resistance depends on the nature of the pathogen and virulence spectra in the pathogen population, the availability, diversity and type of genetic resistance in the host and the methodology for screening and selection for resistance.

During 2010-2012, under the conditions of the infection field of Dobrudzha Agricultural Institute – General Toshevo, Bulgaria, 800 common winter wheat lines were tested to a population of races of the cause agent of leaf rust *P. triticina*. Forty-eight of the lines, which demonstrated resistance under field conditions, were tested to individual pathotypes of the pathogen at seedling stage under controlled conditions. The tested lines, which exhibited a certain degree of resistance at seedling and adult stages, carried both race specific and race nonspecific resistance. The combination of various mechanisms of resistance is highly significant for the durability of resistance. In this relation, the investigated 48 lines can be considered efficient sources of resistance which can be used in the breeding programs.

Key words: *wheat, P. triticina, sources of resistance, race specific resistance, race nonspecific resistance*

Introduction

Leaf rust caused by *Puccinia triticina* Eriks is one of the most common diseases with highest economic importance among the diseases on wheat both in Europe and worldwide. In Bulgaria, the agro climatic conditions are favorable for its development; therefore it occurs annually and influences the yields from wheat. The harmfulness of the disease is expressed mainly in smaller assimilation leaf surface leading to higher transpiration and disturbed hydro balance, which, on its part, leads to premature decay of the leaf mass. The plants damaged by leaf rust typically have low winter resistance and low drought resistance indices. The spikes of the attacked plants form lower amount of grain, which is of poor quality and is the main reason for the lower yields. According to Kolmer et al. (2005), the losses caused by leaf rust can vary from insignificant level to 20 % depending on the growth stage of the crop and the mean resistance or susceptibility of the cultivar. Caldwell et al. (1934) pointed out that the yield decrease in the susceptible cultivars varied within 15-28%, and the losses were mainly due to the reduced number of grains per spike and their lower weight. According to Gospodinova (1979), the losses after early occurrence of the disease can reach 30–40%.

The proper way of solving the problem with leaf rust is developing resistant cultivars and the efforts in this respect are primarily directed to the search and the suitable choice of sources of resistance. The resistance is often neutralized by the occurrence of new pathogen pathotypes. The resistance of the cultivars grown in Bulgaria to *P. triticina* is not sufficient to protect wheat from this pathogen’s attacks for a longer period of time and therefore it is necessary to include new sources of resistance in the breeding programs (Karjin, Stefcheva, 2004).

The aim of this investigation was to study the response of new common winter wheat lines recently developed at Dobrudzha Agricultural Institute – General Toshevo (DAI) to the cause

agent of *P.triticina* at both young and adult stages, and to involve the best of these lines in the breeding for new common winter wheat cultivars.

Materials and methods

During 2010 – 2012, 800 new common winter wheat varieties were screened for resistance to the cause agent of leaf rust *P.triticina* under infection field conditions at DAI. Forty-eight lines were selected out of them, which responded with a certain degree of resistance (moderate MR to high VR) in the infection field, and which demonstrated resistant reaction to some of the leaf rust pathotypes at young stage under greenhouse conditions.

The investigation was carried out under conditions of maximum infection background of *P. triticina.* The full set of pathogen pathotypes of leaf rust identified for the respective year was transferred to field. The lines were sown manually in 1.5 m rows with 25 cm interspacing. Cultivar *Michigan amber* was used as a multiplier of the cause agent of brown rust. The artificial inoculation with the cause agent was done according to a methodology adopted at the Plant Pathology Laboratory of DAI (Ivanova, 2012). The infection type and the attacking rate were read according to Cobb's scale modified by Peterson (Peterson et al., 1948) at stage milk maturity. The mean coefficient of infection (CI) was calculated, or the so called corrected attacking rate, by introducing a coefficient with the following values of the respective infection types: R-0.2; MR-0.4; M-0.6; MS-0.8; S-1. Depending on the values of (CI), the investigated lines were divided into several groups: immune I (CI=0); very resistant VR (CI=0-5.99); resistant R (CI= 6-25.99); moderately resistant MR (CI=26-45.99); moderately susceptible MS (CI=46-65.99), and susceptible to very susceptible S–VS (CI= 66-100). The lines with susceptible reaction (from moderately to very susceptible) were of no interest to us.

The forty-eight lines selected against high infection background which demonstrated a certain degree of resistance, were tested to 9 *P. triticina* pathotypes of various virulence under controlled climatic conditions according to standard procedures (Browder, 1971). The pathotypes were identified on the basis of 15 monogenic lines (*Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr9, Lr11, Lr15, Lr17, Lr19, Lr21, Lr23, Lr24, Lr26* and *Lr28*) and coded by the method of Limpert and Muller (1994). To improve the formation of spores, all plants were treated with solution Maleic hydrazide 97% (1g in 3 l water). On the 9^{th}- 12^{th} day after inoculation, the type of infection was read according to the scale of Stakmen et al. (1962).

Results and discussion

The data from the testing of the investigated lines to 9 pathotypes at young stage are presented in Table 1. The lines demonstrated various degrees of resistance or susceptibility to the pathotypes with different virulence. Line 01/A-169-72 exhibited resistant reaction to all used pathotypes. According to data of (Ivanova, in press), isogenic lines carriers of genes *Lr22a, Lr22b, Lr43* demonstrated complete resistance to all pathotypes at young stage during the three years of the investigation, and only in 2012 genes *Lr9* and *Lr28* also exhibited complete resistance to all pathotypes.

The line indicated above may contain some of these genes but this is a subject of additional genetic and breeding studies. This line responded with high resistance under field conditions during the three years of the investigation (Table 2) which is an indication that it carries race specific resistance.

Table.1 Reaction of common winter wheat lines to 9 pathotypes of *P. triticina* in seedling stage

Cultivar/Lines	Pathotypes of *P. triticina*								
	12773	23773	62572	63562	63567	63573	63773	73763	77562
93/86-9	R	S	S	R	S	R	S	S	R
94/266-3-6	R	S	S	R	R	R	S	S	R
99-10-5-71	S	S	S	R	R	R	S	S	R
99-10-5-72	S	S	S	R	S	R	S	S	S
99/45-83	S	R	S	R	R	R	R	R	R
99/151-9-83	S	S	S	S	S	S	S	S	R
9720-150	S	S	R	S	R	R	R	R	R
9792-115	R	S	S	R	S	R	R	S	R
9792-123	R	S	S	R	S	S	R	S	R
9792-124	S	R	R	R	S	S	R	S	S
9793-126	S	S	S	R	S	S	S	S	S
2534-6-3-4	R	S	S	S	S	R	S	S	R
2969-3-1	S	S	S	S	S	S	S	S	S
3059-1-2	S	S	S	S	S	S	S	S	S
3451-1-3	S	S	S	S	S	R	S	S	R
3506-1-1	S	S	S	S	R	R	S	S	S
3540-4-1	S	S	S	S	S	S	S	S	S
8112-77-11	S	S	S	S	S	S	S	S	S
10337-108	S	R	S	S	R	R	S	S	R
01/A-3	R	S	S	R	R	R	S	S	R
01/A-13	R	S	S	R	S	S	R	S	R
01/A-19-75	R	S	S	R	R	R	S	S	R
01/A-20	R	S	S	R	S	S	S	S	R
01/A-25-73	S	S	S	S	S	R	S	S	S
01/A-29-73	S	S	S	S	S	S	S	S	S
01/A-32-71	S	S	S	S	S	S	S	S	S
01/A-75	S	S	S	S	S	S	S	S	S
01/A-166-73	R	S	S	R	S	R	S	S	R
01/A-169-72	R	R	R	R	R	R	R	R	R
01/A-175-77	R	S	S	R	S	S	S	S	R
01/A-177-72	S	S	S	S	S	S	S	S	S
6A/19-3	S	S	R	R	S	R	R	R	R
20/18-82	S	S	S	S	S	R	S	S	S
20/26	S	S	S	S	S	S	S	S	S
20/27	R	S	S	S	S	S	S	S	S
20/28	S	S	S	R	R	S	S	S	S
20/69	S	S	S	S	S	S	S	S	S
20/93-1	S	S	S	R	S	R	S	S	S
20/96	S	S	R	R	S	R	R	R	R
20/100-5	S	S	R	S	R	R	R	R	S

20/143	S	S	S	S	R	R	R	S	R
20-104-71	S	S	S	S	S	S	S	S	R
20-130-2-71	S	S	S	S	R	S	S	S	S
20/158-77	S	S	S	S	S	S	S	S	S
20/185-74	S	S	S	S	S	R	S	S	S
1/28-84	S	S	S	S	S	R	S	S	S
1/35-85	S	S	S	S	S	S	S	S	S
1/169-87	S	R	R	R	R	R	R	R	R
M. amber	S	S	S	S	S	S	S	S	S

Line 1/169-87 responded with resistant reaction to 8 of the used pathotypes. It demonstrated susceptible reaction only to pathotype 12773. Line 99/45-83 also responded with susceptible reaction to two of the pathotypes (12773 and 62572). The data from Table 2 shows that in 2010 this line reacted as highly resistant to a population of races under field conditions, while in 2011 and 2012 it responded as resistant. Resistant reaction to six of the pathotypes involved in the investigation was demonstrated by lines 9720-150, 6A/19-3 and 20/96. Line 9720-150 reacted as highly resistant under field conditions in all three years of the investigation. The other two lines reacted as resistant in two of the years and as highly resistant in one year. Lines 94/266-3-6, 9792-115, 01/A-3, 01/A-19-75 and 20/100-5 exhibited at young stage resistance to 5 of the used pathotypes. Line 01/A-19-75 demonstrated stable reaction in the three years of the investigation under field conditions reacting as highly resistant. The response of the other four lines to a population of races was within the range very resistance – resistance (Table 2).

Another group of eight lines (99-10-5-71, 93/86-9, 9792-123, 9792-124, 10337-108, 01/A-13, 01/A-166-73and 20/143) exhibited resistant reaction at young stage to four pathotypes of different virulence. Lines 10337-108, 01/A-13 and 01/A-166-73 showed stable highly resistant reaction under field conditions. Stable resistant reaction during the investigated period was demonstrated by lines 93/86-9 and 20/143 (Table 2). The following lines showed resistant reaction at young stage to two or three pathotypes: 99-10-5-72, 3451-1-3, 3506-1-1, 20/28, 20/93-1, 2534-6-3-4, 01/A-20 and 01/A-175-77. Lines 01/A-20 and 20/28 demonstrated high resistant reaction under field conditions, and lines 3506-1-1 and 20/93-1 reacted as resistant. Nine of the studied lines responded with resistant reaction to one pathotype at testing under greenhouse conditions: 99/151-9-83, 9793-126, 01/A-25-73, 20/18-82, 20/27, 20-104-71, 20-130-2-71, 20/185-74 and 1/28-84. All tested lines responded with resistant reaction to one or several pathotypes at seedling stage, and those which demonstrated very resistant and resistant reaction to a population of races under field conditions were carriers of race specific resistance. Further breeding and genetic studies are necessary to find out which genes determine their resistance.

Table 2. Adult plant response

Cultivar/Lines	2010			2011			2012		
	Final rust severity	CI	Rating	Final rust severity	CI	Rating	Final rust severity	CI	Rating
93/86-9	5/4	8.3	R	5/4	8.3	R	10/4	12.5	R
94/266-3-6	10/4	16.7	R	15/4	25.0	R	0	0	VR
99-10-5-71	5/4	8.3	R	0	0	VR	15/4	18.75	R
99-10-5-72	5/4	8.3	R	0	0	VR	5/4	6.25	R
99/45-83	0	0	VR	5/4	8.3	R	5/4	6.25	R
99/151-9-83	0	0	VR	10/4	16.7	R	0	0	VR
9720-150	0	0	VR	0	0	VR	0	0	VR
9792-115	5/4	8.3	R	0	0	VR	0	0	VR
9792-123	0	0	VR	5/4	8.3	R	0	0	VR
9792-124	0	0	VR	5/4	8.3	R	0	0	VR
9793-126	10/4	16.7	R	10/4	16.7	R	0	0	VR
2534-6-3-4	10/4	16.7	R	5/4	8.3	R	5/4	6.25	R
2969-3-1	0	0	VR	0	0	VR	5/4	6.25	R
3059-1-2	0	0	VR	5/4	8.3	R	10/4	12.5	R
3451-1-3	5/4	8.3	R	0	0	VR	5/4	6.25	R
3506-1-1	15/4	25.0	R	5/4	8.3	R	5/4	6.25	R
3540-4-1	5/4	8.3	R	0	0	VR	5/4	6.25	R
8112-77-11	0	0	VR	0	0	VR	5/4	6.25	R
10337-108	0	0	VR	0	0	VR	0	0	VR
01/A-3	0	0	VR	0	0	VR	0	0	VR
01/A-13	0	0	VR	0	0	VR	0	0	VR
01/A-19-75	0	0	VR	0	0	VR	0	0	VR
01/A-20	0	0	VR	0	0	VR	0	0	VR
01/A-25-73	5/4	8.3	R	0	0	VR	0	0	VR
01/A-29-73	5/4	8.3	R	10/4	16.7	R	0	0	VR
01/A-32-71	5/4	8.3	R	10/4	16.7	R	10/4	12.5	R
01/A-75	0	0	VR	10/4	16.7	R	0	0	VR
01/A-166-73	0	0	VR	0	0	VR	0	0	VR
01/A-169-72	0	0	VR	0	0	VR	0	0	VR
01/A-175-77	0	0	VR	5/4	8.3	R	5/4	6.25	R
01/A-177-72	5/4	8.3	R	5/4	8.3	R	5/4	6.25	R
6A/19-3	5/4	8.3	R	0	0	VR	10/4	12.5	R
20/18-82	10/4	16.7	R	10/4	16.7	R	10/4	12.5	R
20/26	5/4	8.3	R	5/4	8.3	R	0	0	VR
20/27	10/4	16.7	R	0	0	VR	0	0	VR
20/28	0	0	VR	0	0	VR	0	0	VR
20/69	10/4	16.7	R	5/4	8.3	R	0	0	VR
20/93-1	5/4	8.3	R	5/4	8.3	R	5/4	6.25	R
20/96	0	0	VR	5/4	8.3	R	5/4	6.25	R
20/100-5	5/4	8.3	R	10/4	16.7	R	0	0	VR
20/143	5/4	8.3	R	5/4	8.3	R	5/4	6.25	R
20-104-71	5/4	8.3	R	0	0	VR	5/4	6.25	R
20-130-2-71	25/4	41.7	MR	0	0	VR	10/4	12.5	R
20/158-77	10/4	16.7	R	10/4	16.7	R	10/4	12.5	R
20/185-74	5/4	8.3	R	5/4	8.3	R	5/4	6.25	R

1/28-84	5/4	8.3	R	5/4	8.3	R	5/4	6.25	R
1/35-85	10/4	16.7	R	5/4	8.3	R	10/4	12.5	R
1/169-87	0	0	VR	5/4	8.3	R	0	0	VR
M. amber	60/4	60.0	VS	60/4	60.0	VS	80/4	80.0	VS

Typical adult or field resistance was exhibited by lines 2669-3-1, 3059-1-2, 3540-4-1, 8112-77-11, 01/A-29-73, 01/A-32-71, 01/A-75, 01/A-177-72, 20/26, 20/69, 20/158-77 and 1/35-85. All these lines responded with susceptible reaction to all pathotypes used at young stage, while demonstrating resistant to very resistant reaction in field. The resistance these lines carry is race-nonspecific.

Conclusion

All tested lines responded with resistant reaction to one or several pathotypes at seedling stage, and those with very resistant and resistant reaction to a population of races under field conditions were carriers of race specific resistance. Line 01/A-169-72 demonstrated complete resistance to all pathotypes used at young stage. This line could carry genes *Lr22a, Lr22b, Lr43, Lr9* or *Lr28* since these isogenic lines responded with resistant reaction to the used pathotypes; further genetic and breeding investigations are however necessary to confirm this.
A part of the lines, 2669-3-1, 3059-1-2, 3540-4-1, 8112-77-11, 01/A-29-73, 01/A-32-71, 01/A-75, 01/A-177-72, 20/26, 20/69, 20/158-77 and 1/35-85, carried race nonspecific resistance.
Every breeding strategy should include a rich set of sources with different types and mechanisms of resistance to allow both genetic variability and alternatives for correction of the “erosion of race specific genes” phenomenon. On the other hand, the combination of resistant genes at young stage with APR genes expresses a high level of durable rust resistance. In this sense the investigation carried out can assist breeding and the presented lines can be involved in the breeding programs.

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THE ROLE OF PLANT GROWTH REGULATORS ON PHYSIOLOGICAL PARAMETERS IN WINTER WHEAT UNDER DROUGHT STRESS

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Abstract

Drought is the most important environmental factor that adversely affects plant stands. Application of growth regulators, we can achieve a partial elimination of the impacts of environmental stress. The aim of this study was to determine the impact of growth regulators on the physiological parameters, grain yield and quality of winter wheat under drought stress simulated by experimental rain-out shelters. We hypothesized that growth regulators can contribute to mitigating the impact of drought on water use efficiency, yield formation and grain quality of winter wheat production. The experiment was conducted in a field experimental station in Žabčice (49°00'41.3"N) on winter wheat variety Matylda in 2013/2014. The experimental station is located in a warm area with prevailing continental climate (average annual rainfall 482 mm and temperature 9.3°C). Within this experiment following growth regulators and fungicide with growth regulation effect were used: Retacel extra R68 (chlormequat chloride 720 g/l), Moddus (trinexapac-ethyl 250 g/l), Cerone (ethephon 480 g/l), Amistar (azoxystrobin 250 g/l). These growth regulators were applied at growth stages between BBCH 31 and BBCH 49. Application of growth regulators partly eliminated negative impact of drought on chlorophyll fluorescence, chlorophyll and flavonol content and grain yield particularly for azoxystrobin, chlormequat chloride and trinexapac-ethyl applications. Growth regulators also reduced the negative impact of drought on grain quality. Results of field experiments can contribute to mitigating the impact of drought on yield formation of winter wheat production in the realization of biological potential of wheat genotypes.

Keywords: *winter wheat, drought, growth regulators, physiological parameters, yielde*

Introduction

Wheat is one of the most important crops all over the world. Worldwide production of wheat increasing every year, and the increase is a result of higher yields and slightly larger area of all crops, which may reach 222.3 million hectares. Drought stress in winter wheat is evidently more significant in the Central Europe than in other countries, it is also supposed that according to the model of climate prediction periods of drought are going to be more often. Drought decreases the growth of plants, influences various physiological and biochemical processes such as photosynthesis, respiration, metabolism of nutrients and growth stimulators. Drought is one of the most common environmental stresses that affect growth and development of plants.Tolerance to water stress is a complicated parameter in which crops performance can be influenced by several characteristics (Ingram and Bartels, 1996). Tolerance can be divided into two parts including drought avoidance and dehydration tolerance (Kramer et al., 1995). Drought avoidance includes root depth, reasonable use of available water by plants, and changes in plants' lifestyle to use rainfall. Dehydration tolerance consists of plants' capability to partially dehydrate and grow again when rainfall continues (Salekdeh et al., 2002). Adaption of plants to drought stress is a vital issue to develop new improve methods for increasing stress tolerant plants (Rizhsky et al., 2002).

Many factors can affect plants' responses to drought stress such as plant genotype, growth stage, severity and duration of stress, physiological process of growth (Chaves et al., 2003), different patterns of genes expression (Denby et al., 2005), different patterns of the activity of respiration(Ribas-Carbo et al., 2005), activity of photosynthesis machinery (Flexas et al., 2004), and environmental factors (McDonald, 1996). Physiological responses include closure of stomata, decrease in the activity of photosynthesis, development of oxidative stress, alteration in the integrity of cell wall, production of metabolites which are toxic and cause plants' death (Bray, 2002), signal recognition of roots, turgor loss and adjustment of osmosis, reduction in water potential of leaf, decrease in stomata conductance to CO2, reduction of internal CO2 concentration, and reduction of growth rates.Photosynthesis is an essential process to maintain crop growth and development, and it is well known that photosynthetic systems in higher plants are most sensitive to drought stress (Falk et al., 1996). The effect of water stress on photosynthesis has been a subject of controversy among plant physiologists for many years, and conflicting results have been reported depending on the plant material, and the experimental procedures used for investigations (Comic and Massacci, 1996). However, it has not been well established that where and how the chloroplasts are damaged underdrought stress, and how the damages can be detected and evaluated easily. In response to water stress, a decrease in net CO, assimilation is generally observed. This effect can result from different events, such as an inhibition of electron transport activity limiting the generation of reducing power or a limitation in the metabolic activity (Guo and Al-Khatib, 2003). Classical methods, based on CO, and water exchange measurements, provide information concerning net photosynthetic activity; however, these informations are not sufficient, and additional techniques are required to determine without ambiguity the effects of water stress on photosynthesis. It was reported that the approach for measuring photosynthetic traits such as chlorophyll content and chlorophyll fluorescence parameters might estimate influence of the environmental stress on growth and yield, since these traits were closely correlated with the rate of carbon exchange (Guo and Li, 2000; Araus et al., 1998; Fracheboud et al., 2004). So these parameters can be used as reliable indicators to evaluate the energetic/metabolic imbalance of photosynthesis and yield performance across genotypes under water deficit (Araus and Hogan 1994; Araus er al. 1998). Unfortunately, little is known about changes of these traits such as chlorophyll content and chlorophyll fluorescence parameters under drought stress in wheat. In this study, dry and wet varietes differing in drought tolerance were selected to compare chlorophyll and flavonolcontent and chlorophyll fluorescence parameters under drought stress. The relationship between these traits and drought tolerance is discussed in this study.

Material and methods

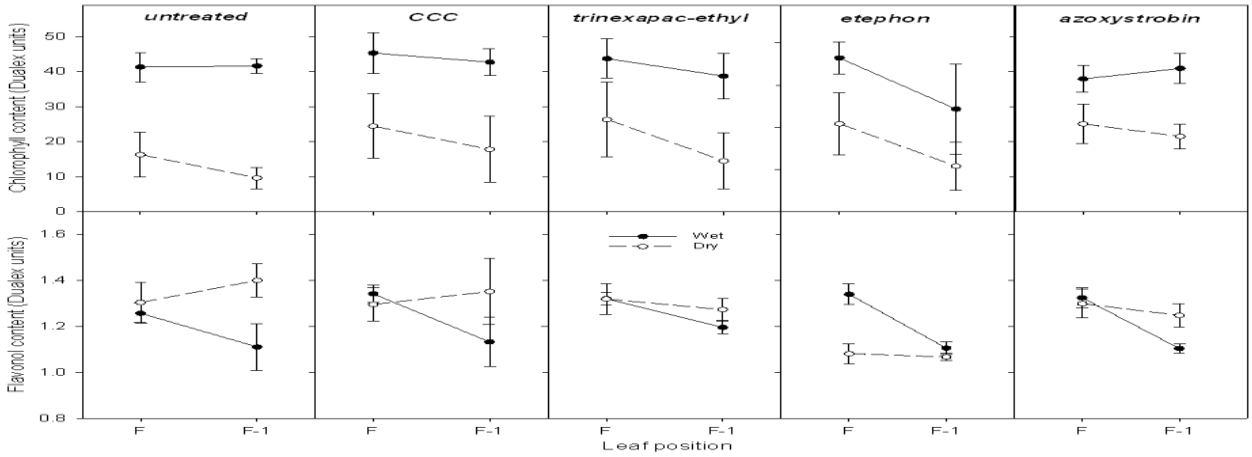
The experiment was carried out at the field experimental station in Žabčice with winter wheat variety Matylda. The experimental station is situated in Southern Moravia (the Czech Republic). Moderate soils are dominant type in this region. The location is considered to be one of the hottest areas in the Czech Republic. Sowing of the variety Matylda was carried out on October 15th, 2013 in three replications randomly distributed on selected plot with sowing rate of 4 million growing seeds/ha. Variety Matylda belongs to the set of early varieties. The variety has a medium plant height with an average resistance to lodging. Variety Matylda has a very high yield potential. During the growth phase by the end of stem elongation period BBCH 39 there were over the half of the experimental area built short-termed rain out shelters providing induction of drought stress. Measuring of physiological parameters (water use efficiency, CO_2 assimilation rate and the chlorophyll content in leaves was done in the middle of drought stress (May 26th, 2014), and at the end of drought stress effect.After wheat ripening evaluation of yield and yield structure has been done. For evaluation of CO_2 assimilation rate,

transpiration and stomatal conductivity gas exchange system LI 6400 XT with an assimilation chamber equipped with LED light source has been used. The measurement took place at constant temperature, relative air humidity, CO_2 concentration and at saturation light intensity. The observed parameters allowed calculation of water use efficiency (WUE) and indirect parameter of water use efficiency A/Gs.As an additional parameter for evaluation of primary phase of photosynthesis measuring of chlorophyll fluorescence by the apparatus FluorPen has been done. Parameters of the maximal and actual quantum yield PS II were evaluated. The content of chlorophyll and flavonols was determined in vivo by the method of transmittance and UV screening of chlorophyll fluorescence by the instrument Dualex4 FLAV.

Results and discussion

Chlorophyll and flavonol content

Drought stress led to a general decline in chlorophyll content in both upper leaves (F and F-1) (Graph 1). All growth regulators used in the experiment reduced this decline, particularly in the flag leaf. The highest mitigating effect on drought caused decline in chlorophyll content was observed for active ingredient azoxystrobin. Active ingredient etephon reduced negative effect of drought on chlorophyll content, but also led to a decrease in chlorophyll content in lower leaf (F-1) (Graph 1), both in the treatment well watered and drought stressed. Conversely, the flavonoid content in leaves of plants exposed to drought stress increased particularly in the lower leaf (F-1). Growth regulators generally reduce this effect, while the most significant effect was found for application of etephon where flavonoid content in drought stressed plants dropped below a level of well watered plants.

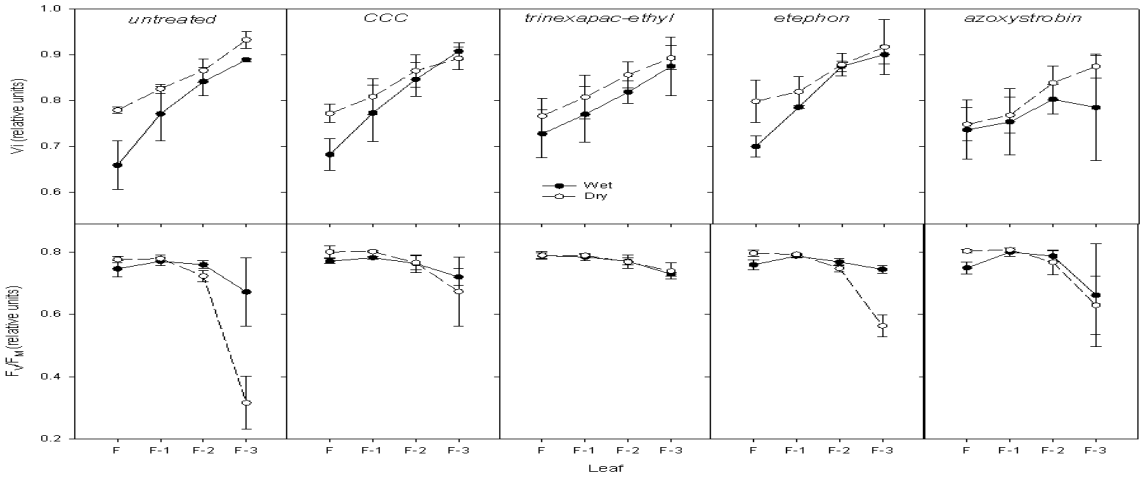


Graph 1: Chlorophyll and flavonol content

Chlorophyll fluorescence

In the term of measurements, chlorophyll fluorescence parameters V_i and F_V / F_M were affected differently within the vertical canopy profile. V_i parameter shows response to drought stress in virtually all leaves within the vertical canopy profile, while the values increased in direction to the lower leaves. Conversely, a parameter F_V/F_M was significantly affected by drought stress only in the lowest leaf (F-3) (Graph 2). The positive effect of growth regulators on partametr V_i occurred only in the lower leaves, particularly for growth regulators CCC and etephon. Conversely, the positive effect of regulators reducing the impact

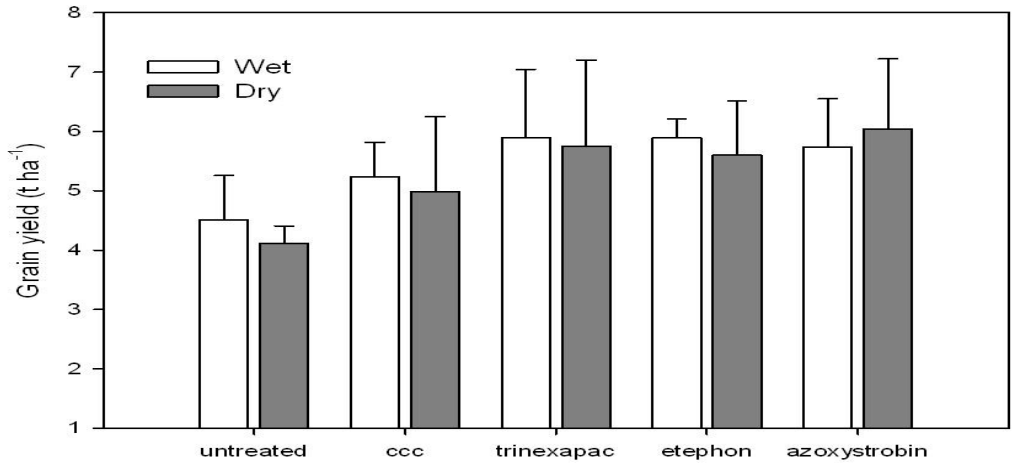
of drought on the parameter F_V/F_M was seen in all growth regulator treatments with the most significant effect of the active ingredience trinexapac-ethyl.



Graph 2: Chlorophyll fluorescence

Grain yield

Yield results showed a positive effect of all applications of growth regulators, with the highest impact of the trinexapac-ethyl, etephon and azoxystrobin. All three treatments also reduced the negative impact of drought on yield....Graph 3



Graph 3: Grain yield

Conclusions

In this study, we monitored the values of chlorophyll and flavonol content, Fo, FV/Fo and FV/F, under watered and drought stress condition.The highest mitigating effect on drought caused decline in chlorophyll content was observed for active ingredient azoxystrobin.Conversely, the flavonoid content in leaves of plants exposed to drought stress increased particularly in the lower leaf (F-1). Growth regulators generally reduce this effect, while the most significant effect was found for application of etephon where flavonoid content in drought stressed plants dropped below a level of well watered plants.Conversely, the positive effect of regulators reducing the impact of drought on the parameter F_V/F_M was seen in all growth regulator treatments with the most significant effect of the active ingredience trinexapac-ethyl.It is evident from the measurements that all the regulators increased the yield and which it itself proves the positive effect during the drought because it

was a very dry year and the canopy did not lodge. Furthermore, the biggest reduction of drought stress effect on yield was evident in strobilurin and partially at trinexapac-ethyl application as well.Therefore, these photosynthetic traits could be good indicators of adaptation of wheat to drought stress. Since the measurement for these traits and using plant growth regulators is fast and reliable, the approach is quite attractive.

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EFFECT OF SOME CITRUS ROOTSTOCKS ON YIELD AND FRUIT QUALITY OF TWO MANDARIN VARIETIES

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Abstract

The present study was carried out during the two successive seasons of 2013 and 2014 to investigate the effect of volkameriana lemon (VL) and sour orange (SO) rootstocks on yield and fruit quality of Balady and Fremont mandarin cultivars. Generally rootstocks were found to have significant effects on yield, and fruit quality of both varieties. Balady and Fremont mandarin trees budded on VL rootstock showed higher fruit yield/ tree (kg) fruit weight(g), volume(cm^3), height, diameter (cm), fruit pulp weight (g), fruit peel weight (g) and peel thickness (cm), juice volume of fruit (ml), number & weight of seeds/ tree and fruit firmness than those dudded on SO rootstock. On the other hand, trees budded on SO rootstock had the highest of TSS%, total acidity of fruits juice and vitamin C compared with those budded on VL rootstock. Oppositely, the effect of the rootstocks on TSS/acid ratio of two scion cultivars was found to be insignificant. Fremont mandarin cultivar was the higher in yield/ tree, fruit weight, pulp weight, fruit juice, acidity and vitamin C than Balady one, Balady cv. was superior to Fremont mandarin regarding the other characteristics. Therefore, it was found that VL is a suitable rootstock for Balady and Fremont mandarin cultivars compared with SO.
Key words: Citrus, mandarin, rootstock, yield and fruit quality.

Introduction

Citrus is the first fruit crop in Egypt whereas, it is covering an area of 395731feddan with annual production of 2786397tons (**FAO, 2012**). Mandarin occupies the second planted citrus species after orange with 27.2 % of citrus cultivated area and 21.7 % of total citrus production (**The Management of Central Horticulture, 2011**).Rootstocks have had a substantial role in the development of the citrus industry in the world. Rootstock utilization has gain value for solving both limiting and restricting factors of citrus production (soil, climate and pests, etc.) and conditioning the market demands on productivity, short juvenility period and high-fruit quality **(Tuzcu et al., 1992).**Factors like cultivar characteristics, rootstocks employed, growing conditions along with cultural managements, type of flowers, and the fruit drops can affect citrus cultivars yield and quality performance **(Demirkeser et al., 2003).**The rootstock may influence several aspects of citrus growth and development, including yield, fruit quality, and tolerance to stress caused by biotic and abiotic factors **(Filho et al., 2007).** In the Mediterranean region, all citrus cultivars are mainly budded on Sour orange, due to its resistance to gummosis fungi, high adaptability to wide range of soil conditions and the ability to produce high fruits quality (**Castle, 2010**).However, the Sour orange rootstock has shown to have some serious problems such as susceptibility to be attacked by citrus tristeza virus, poor compatibility with some citrus cultivars and in some cases produce low fruit yields compared with other rootstocks (**Castle, 2010 and Georgiou, and Gregoriou, 1999**).Volkameriana lemon which appears to be one of the most promising rootstocks because its tolerance to tristeza (**Shafieizargar et al., 2012**).Balady and Fremont mandarin cultivars are growing in new reclaimed soils and suffering from the aforementioned problems. Accordingly, this experiment was conducted to elucidate the effect of two rootstocks on yield and fruit quality of two mandarin cultivars.

Materials and methods

The present study was carried out in the two successive seasons of 2013 and 2014 where trees of Balady and Fremont mandarin (***Citrus reticulate Blanco***) of eight years old budded on Sour orange (SO) (***C. aurantium*** L.) and Volkamer lemon (VL) (***Citrus volkameriana*** **Ten. and Pasq.**), rootstocks. The trees were planted at 3x5 meters apart for Balady and 1.5x5 for Fremont mandarin trees and grown in a private orchard located at Alsalehia Algadeeda, Alsharkia Governorate, Egypt, were used. The trees were grown in sandy soil, irrigated using drip irrigation system. Fifteen trees of each rootstock and Mandarin cultivar were selected nearly similar in growth, healthy and subjected to the same cultural practices, where it divided into three replicates, with five trees per each replicate. Ninety fruits from each cultivar budded on every rootstock were harvested at fruit at maturity stage, where 6 fruits of each tree were used. The collected fruits were divided into three replicates for each rootstock and Mandarin cultivar.

Measurements:

Yield: Harvesting was achieved on 10thFebruaryand 15th December of Balady and Fremont cvs. respectively and fruits per tree were collected and separately weighed so that the yield per tree (kg) could be calculated in both studied mandarin cultivars and in two studied seasons.

Fruit physical and biochemical characteristics: Samples of 90 fruits were collected at maturity stage from 15 trees such that 6 fruits per tree. The harvested fruits were divided into three replicates in each mandarin cultivar budded on either SO or VL rootstocks to determine some fruit physical and biochemical characteristics.

Physical characteristics such as fruit weight (g), fruit volume (cm^3), fruit pulp weight (g), fruit peel weight (g) and peel thickness (cm) juice volume of fruit (ml), number and weight of seeds/fruit were recorded. Fruit firmness expressed as Newton (N) was measured using pressure tester (digital force-Gouge ModelIGV-O.SA to FGV-100A.Shimpo instruments).

Fruit biochemical characteristics. The following characteristics were recorded as follows:

Total soluble solids (TSS %) was determined by using a digital refractometer.

Total acidity (TA) was determined by titration and expressed as citric acid according to **A.O.A.C., 2000**.

Total soluble solids/acid ratio was calculated from the values of total soluble solids divided by values of total acids. Also, **Ascorbic acid (VitaminC)** was estimated by titrating juice sample with 2, 6 dichlorophenol indophenol dye according to **A.O.A.C., 2000**.

Statistical Analysis: A complete randomized block design was followed and the analysis of variance (ANOVA) was performed using two ways ANOVA Co-stat software according to **Stern (1991),** and the means were differentiated using Duncan multiple range test at 5% level **(Duncan, 1955).**

Results and discussions

The yield

Yield was affected by the kind of rootstock in both scion cultivars, in the two studied seasons (Fig.1). Trees of the two cultivars budded on VL possessed yield significantly higher than those budded on SO in the two studied seasons. Data also revealed that Fremont cultivar gained fruit yield/tree significantly higher than those of Balady cv. which possessed the least values in the two tested seasons.

The present results are in agreement with that previously obtained by **Hifny et al., (2013)** on Valencia orange, **Shafieizargar, et al., (2012)** on Queen orange and **EI-Sayed et al., (2007)** on Washington navel who found that orange trees budded on VL produced fruit yield (kg/tree) higher than those budded on SO rootstock. Further, **Filho et al., (2007)** mentioned that fruit yield of ‘Fallglo’ and ‘Sunburst’ mandarin trees was not affected by the rootstock. These

results indicated the inconsistency in yield differences as affected by rootstocks, which could be attributed to differences in scion cultivars, tree age, climatic conditions, and soil characteristics, etc.**(Yildiz1 et al.,2013).**The lower yield efficiency on sour orange and ‘Carrizo’ is probably caused by the bigger canopy size induced by these rootstocks. The higher yield efficiencies were also reported for trees reduced in size by rootstocks **(Auler et al., 2008).**Yield difference among rootstocks and their interactions with different citrus cultivars could be attributed to differences in morphology and physiology of rootstocks, which are reflected as tree growth vigour, size and depth of roots, water and nutrients uptake capability, carbohydrate synthesis, and also their adaptation to climatic and soil conditions, good compatibility between rootstock and cultivar and the possibility of fruiting potential of a cultivar on certain rootstocks **(Continella et al., 1998)**. The compatibility between rootstock and scion is very important to achieve sufficient yield. These various characteristics of rootstocks can affect growth, fruiting, and fruit quality of the scion cultivar. VL rootstock usually supplies the scion with an extreme vigor, great tree size, and cause large fruit. VL rootstock vigority may be attributed to its ability to generate a more extensive root that would absorb more water and nutrients **(Reuther et al., 1967).**Sour orange, the most common citrus rootstock, produced high quality fruits but smaller sized fruits out of marketable grade. In contrast, the ‘Volkamer’ lemon produced good quality marketable sized moderate number of fruits and proved as a reliable rootstock for ‘Queen’ orange**(Shafieizargar, et al., 2012)**.

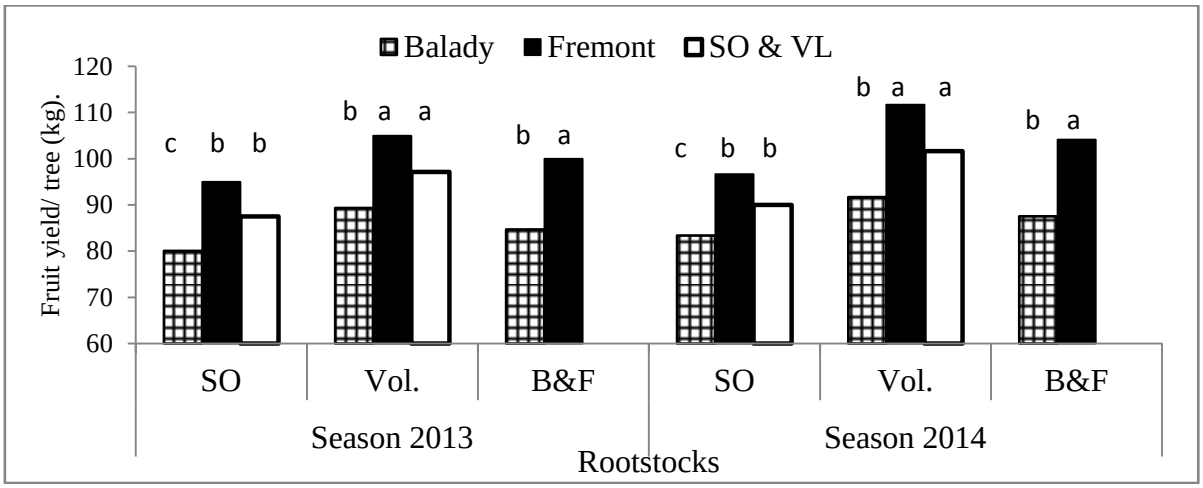

Fig.1. Effect of citrus rootstocks on the yield of Balady and Fremont mandarin cultivars 2013 and 2014in seasons.

Fruit physical characteristics.

Data in Table (2) showed the effect of Sour orange (SO) and Volkamer lemon (VL) citrus rootstocks on fruit weight (g), volume (cm^3), peel weight (g),pulp weight (g),fruit height(cm), diameter(cm) , Peel thickness (cm), juice volume (cm^3), number of segments/ fruit, number of seeds/ fruit, weight of seeds/ fruit and firmness of both Balady and Fremont mandarin cultivars in the two studied seasons.

Regarding the effect of rootstock kind on fruit weight and fruit volume of Balady and Fremont mandarin, fruits the results in table (2) showed that fruit weight, fruit volume (cm^3), fruit peel weight (g) and fruit pulp weight (g) of both Balady and Fremont mandarin cvs. budded on VL was significantly bigger than those budded on SO. in two studied seasons. Fremont cv. had the higher fruit weight, fruit volume and fruit pulp weight than Balady one while Balady cv. gained the highest fruit peel weight in two studied seasons.

The results are in agreement with those of **Hifny et al., (2013)**who found that fruit weight of Valencia cv. budded on VL was greater than those budded on SO. **Al-Jaleel and Zekri (2002)**

found that fruit weight of Olinda Valencia budded on VL was higher than those budded on SO rootstocks. On the other hand, **Filho et al. (2007)** on Fallglo and Sunburst mandarins reported that fruit weight was not affected by the rootstocks. **Shafieizargaret al., (2012)**suggested that the fruit size of Queen orange was highly affected by many growth factors such as nutrients and water availability and extensive rooting system, it could have contributed to the bigger fruits of trees on VL and other vigorous rootstocks.

Concerning the effect of rootstock kind on fruit height, fruit diameter and fruit Peel thickness (cm) data in table (2) showed significant increase in fruit height, diameter and Peel thickness (cm) of Balady and Fremont mandarin trees budded on VL compared with those budded on SO rootstocks. Data in table (2) also illustrated no a real significant difference between both studied cvs. was noticed regarding fruit height and diameter while significant difference between them was noticed regarding fruit peel where Balady cv. Possessed higher fruit Peel thickness (cm) than Fremont one in the two studied season.

The results are in agreement with those of **Yildiz1 et al., (2013)** reported that higher values of fruit diameter were verified in fruits of Rhode Red Valencia orange trees on Carrizo and Troyer rootstocks; however Valencia Late on Carrizo rootstock had the least fruit diameter. The highest rind thickness was also obtained in fruits of Rhode Red Valencia on Troyer and those of Valencia Late on Carrizo and Troyer rootstocks. **Incesu et al.,(2013)** found that maximum fruit length of Moro blood orange was highest obtained from fruit on Yuzu and the lowest one on Troyer citrange.

Regarding the effect of kind of rootstock on fruit juice volume, data in table (2) showed significant increase in fruit juice volume (cm^3) of Balady and Fremont mandarin trees budded on VL compared with those budded on SO rootstocks. Balady cv. had the higher fruit juice volume (cm^3) than Fremont cv., in the two studied season.

These findings are in harmony with those of **Incesu et al.,(2013)** found significant difference between the effect of rootstocks on fruit Juice of Moro blood oranges. **Ahmad et al., (2007)** on Kinnow mandarin found that the highest juice content was with VL rootstock. On the other hand of **Yildiz1 et al., (2013)**mentioned that no significant difference between the effect of tested rootstocks on fruit Juice of Rhode Red Valencia and Valencia Late orange.

Data in table (2) indicated that number and weight of seeds significantly increased of Balady and Fremont mandarin fruits when budded on VL compared with those budded on SO rootstocks. Balady cv. gained the highest number and weight seeds / fruit compared with Fremont cv., which possessed the least values in the two studied season.

The results are in agreement with those of **Demirkeser et al., (2009)** who found that the number of seeds was significantly influenced by the rootstocks in Nova and Robinson fruits where the trees were grafted on SO rootstock higher than which was grafted on Troyer rootstock. However, seed number did not reach commercially unacceptable levels. On the contrary **Ghnaim and Al-Muhtaseb, (2006)** working on Washington Navel found that no significant differences for seed number were observed among the four rootstocks.

Regarding the effect of kind of rootstock on Pulp Firmness (N), data in Table (2) showed significant increase in pulp firmness of Balady and Fremont mandarin fruits budded on VL compared with those budded on SO rootstocks. Balady cv. had the higher Pulp Firmness than those of Fremont cv., in the two studied season.

The results are in agreement with those of **Hifny et al., (2012)**who found that pulp firmness of Valencia cv. budded on VL was higher than those budded on SO. Similar results were gained by **Wutscher and Bistline(1988**) who stated that the little percentage of fruit juice caused by budding on VL rootstock has the responsible for the higher pulp firmness compared with that on SO rootstock.

Fruit biochemical characteristics.
Data in Table (3) showed the effect sour orange (SO) and Volkamer lemon (VL) citrus rootstocks on the percentages of total acidity, TSS , TSS/ Acid ratio and Vitamin C (Ascorbic Acid) of Balady and Fremont mandarin fruits in the two studied seasons.
Total Soluble Solids (TSS %). Data in Table (3) cleared that total soluble solid of fruits from trees budded on SO rootstock had higher TSS % compared with those budded on VL rootstocks. Balady cv. possessed higher TSS % values than Fremont cv., in the two studied season. The results are in agreement with those of **Al-Jaleel et al.,(2005)**who found an increase in TSS% in fruit juice of Allen Eureka lemon scions budded on VL rootstock compared with SO rootstock. **Kaplankiran et al., (2005)** working on Okitsu Satsuma mandarin reported that the highest TSS was obtained from fruits on sour orange. Generally, some vigorous rootstocks such as VL and rough lemon, which normally have an extensive rooting system, are able to absorb more water and produce heavy crop loads with higher juice content but this is sometimes coupled with low TSS. Some other rootstocks such as Sour orange and 'Cleopatra' mandarin produce fruits with lower juice content but with high TSS **(Reuther et al., 1967).**
Total Acidity. Data in Table (3) indicated that the lowest total acid percentage was gained in fruits budded on SO rootstock with significant differences than VL rootstock. Fremont cv. had the higher total acidity percentage than Balady cv., in the two studied season. The results are in agreement with those of **Hifny et al., (2013)** who noticed an increase in total acidity in fruit juice of Valencia scions budded on SO rootstock compared with those budded on VL rootstock. Oppositely **Incesu et al.,(2013)** on Moro blood oranges reported that the effects of the rootstocks on fruit juice acidity were insignificant.
TSS/Acid Ratio. Data in Table (3) indicated that TSS/Acid ratio of fruit juice did not show a significant difference between the effects of two studied rootstocks. While Balady cv. had the higher TSS/Acid ratio in fruit juice than Fremont cv., in the two studied season. The maximum values of TSS/Acid ratio in fruit juice was recorded in fruits budded on SO rootstock compared with those budded on VL rootstock. The flavor and palatability of citrus fruit is a function of relative levels of TSS%, acids and the presence or absence of various aromatic or bitter juice constituents **(Davies and Albrigo, 1994).** The results are in agreement with those of **Incesu et al.,(2013)** on Moro blood oranges who found that the TSS/TA ratio, is generally defined as the fruit maturity index in citriculture, was not affected by rootstocks. Oppositely **Hifny et al., (2013)** found an increase in TSS/Acid ratio in fruit juice of Valencia scions budded on VL rootstock compared with those budded on SO rootstock.
Vitamin C (Ascorbic Acid). Data in Table (3) cleared that fruits from trees budded on SO rootstock showed an increase in Vitamin C content significantly higher than those on VL rootstock. Fremont cv. had the higher of vitamin C in fruits juice than Balady cv., in the two studied season. The results were in harmony with those obtained by with **Hifny et al., (2013)** who reported that the ascorbic acid (V.C.) of Valencia orange fruit trees budded on SO rootstock was superior to those budded on VL rootstock. On the contrary **EI-Sayed et al. (2007)** found that Washington navel orange budded on VL, produced fruits with highest juice acidity and ascorbic acid but presented lower TSS and TSS/acid ratio at harvest time in both seasons. The increase in Ascorbic acid (V.C. mg/100ml of fruit juice) as well as Titratable acidity percentage in fruit juice at harvest time could be attributed to the increase in fruit weight and size and to the increase in shoot growth and leaf area caused by N application **(Chen et al., 1999).** According to several researches, citrus trees grafted on Sour orange rootstock can be expected to produce medium-sized to large fruit with high Total Soluble Solids (TSS %) and high juice acidity **(Ramin and Alirezanezhad, 2005; and Zekri and Al-Jaleel, 2004)**. On the other hand, Citrus trees grafted on lemon rootstocks are usually expected to produce larger fruits with poor fruit quality; thick rinds, low total soluble solids

and low juice acidity **(Zekri and Al-Jaleel, 2004) .**

Conclusion

The study revealed that rootstocks produced marked effects on most of the parameters measured; suggesting that tree size, yield, and fruit quality of both Balady and Fremont mandarin cvs. can be controlled by proper selection of rootstock. Trees budded on VL rootstock showed the highest fruit yield/ tree (kg) fruit weight, volume, height, diameter, fruit pulp weight, fruit peel weight and peel thickness, juice volume of fruit (ml), number & weight of seeds/ tree and fruit firmness compared with the trees duded on SO rootstock. While trees budded on SO rootstock had the highest TSS%, total acidity of fruits juice and vitamin C. Therefore, it was found that VL is a suitable rootstock for both Balady and Fremont mandarin cultivars.

Table 2: Effect of two citrus rootstocks on some physical characteristics of Balady andFremont mandarin cultivars in2013 and 2014seasons.

Characters	Fruit weight (g)		Means B	Fruit volume (cm^3)		Means B	Fruit peel (g).		Means B	Fruit pulp (g).		Means B
Rootstocks / Cultivar	SO.	VL.		SO.	VL.		SO.	VL.		SO.	VL.	
	Season 2013											
Balady	108.33 c	119.09 b	113.71 b	108.33 bc	136.04 a	122.19 a	38.21 a	37.55 a	37.88 a	70.13 c	81.54 b	75.84 b
Fremont	116.13 bc	128.60 a	122.37 a	104.22 c	112.50 b	108.36 b	27.67 c	33.22 b	30.44 b	88.47 ab	95.38 a	91.93 a
Means A	112.23 b	123.85 a	------	106.28 b	124.27 a	------	32.94 b	35.38 a	----	79.30 b	88.46 a	----
	Season 2014											
Balady	115.56 c	128.57 ab	122.07 b	119.07 b	125.47 a	122.27 a	35.33 a	33.80 a	34.57 a	80.23 b	94.77 a	87.50 b
Fremont	124.73 bc	138.43 a	131.58 a	111.67 c	120.50 b	116.08 b	29.07 b	35.51 a	32.29 b	95.67 a	102.9 a	99.30 a
Means A	120.15 b	133.50 a	-----	115.37b	122.98 a	-----	32.20 b	34.66 a	-----	87.95 b	98.85 a	-----

Means followed by the same letter (s) in each column or row and the interactions are not significantly different at 5 % level.

Table (2) Continue:

Characters	Fruit height (cm)			Fruit diameter (cm)			Peel thickness (cm)			Juice volume(cm^3)		
Rootstocks / Cultivar	SO.	VL.	Means B	SO.	VL.	Means B	SO.	VL.	Means B	SO.	VL.	Means B
	Season 2013											
Balady	5.13 b	5.29 a	5.21 a	6.21 bc	6.59 a	6.40 a	0.43 b	0.45 a	0.44 a	41.33 c	42.33 c	41.83 b
Fremont	5.10 b	5.33 a	5.22 a	6.10 c	6.30 b	6.20 b	0.35 d	0.40 c	0.37 b	57.00 b	62.67 a	59.83 a
Means A	5.12 b	5.31 a	-----	6.16 b	6.45 a	-----	0.39 b	0.43 a	----	49.17 b	52.50 a	----
	Season 2014											
Balady	5.20 b	5.47 a	5.33 a	6.10 c	6.74 a	6.42 a	0.41 a	0.42 a	0.42 a	38.67 c	37.67 c	38.17 b
Fremont	5.23 b	5.50 a	5.37 a	6.20 c	6.46 b	6.33 a	0.33 c	0.39 b	0.36 b	50.33 b	61.00 a	55.67 a
Means A	5.22b	5.48 a	------	6.15b	6.60 a	-----	0.37 b	0.41 a	---	44.50 b	49.33 a	-----

Table (2) Continue:

Characters	Number of seeds/ fruit			Seed weight(g)/fruit			Firmness (N)		
Rootstocks / Cultivar	SO.	VL.	Means B	SO.	VL.	Means B	SO.	VL.	Means B
	Season 2013								
Balady	13.67 b	25.17 a	19.42 a	1.33 b	4.03 a	2.68 a	24.53 b	29.33 a	26.93 a
Fremont	12.00 b	10.67 b	11.33 b	1.58 b	1.42 b	1.50 b	21.64 c	22.31 bc	21.98 b
Means A	12.83 b	17.92 a	----	1.46 b	2.73 a	-----	23.09 b	25.82 a	-----
	Season 2014								
Balady	12.67 b	22.00 a	17.33 a	1.20 b	3.03 a	2.12 a	22.31 b	24.25 a	23.28 a
Fremont	11.33 b	10.00 b	10.67 b	1.36 b	1.32 b	1.34 b	19.99 c	20.81 bc	20.40 b
Means A	12.00 b	16.00 a	----	1.28 b	2.18 a		21.15 b	22.53 a	------

Table 3: Effect of citrus rootstocks on some chemical characteristics of Balady and Fremont mandarin cultivars in2013 and 2014seasons.

Characters	TSS %			Acidity %			TSS/Acid ratio			V.C(mg Ascorbic acid/ 100 ml of fruit juice)		
Rootstocks / Cultivar	SO.	VL.	Means B	SO.	VL.	Means B	SO.	VL.	Means B	SO.	VL.	Means B
	Season 2013											
Balady	13.67 a	11.17 c	12.42 a	1.12 a	0.94 c	1.03 b	12.24 a	11.88 a	12.06 a	36.67 b	30.00 c	33.33 b
Fremont	12.50 b	10.33 d	11.42 b	1.19 a	1.03 b	1.11 a	10.52 b	10.03 b	10.27 b	45.33 a	38.00 b	41.67 a
Means A	13.08 a	10.75 b	----	1.15 a	0.99 b	----	11.38 a	10.96 a	-----	41.00 a	34.00 b	----
	Season 2014											
Balady	14.33 a	11.67 c	13.00 a	1.10 ab	0.95 c	1.03 b	12.99 a	12.3 ab	12.62 a	41.7 ab	35.33 b	38.5 b
Fremont	12.83 b	11.00 c	11.92 b	1.17 a	1.06 b	1.11 a	11.0 bc	10.41 c	10.72 b	48.00 a	41.0 ab	44.5 a
Means A	13.58 a	11.33 b	-----	1.14 a	1.01 b	-----	12.01 a	11.33 a	-----	44.83 a	38.17 b	-----

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EFFECTS OF WATER DEFICIT ON GROWTH PARAMETERS OF TWO POPULATIONS OF LOTUS CORNICULATUS

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Abstract

Water deficit is the most severe limitation factor of plant growth and development, while plant genotypes may express different water stress hardiness. In the present study, *Lotus corniculatus* seedlings of two different populations (Kilkis and Taxiarchis; northern Greece) were used to evaluate the effect of water deficit on growth characteristics. Plants from the two populations were transplanted to pots and following a two-month establishment period, they were subjected to two water regimes: a) irrigation up to field capacity and b) limited irrigation in order to maintain water deficit conditions in the soil. Stem weight (SW), leaf weight (LW) and leaf area (LA) were measured during the growing season at four different phenological stages. The allometric indices of specific leaf area (SLA), leaf area ratio (LAR) and leaf water content (LWC) were estimated. Our findings suggest that plant origin (population) is a significant predictor of SW, LW, LA, SLA, LAR and LWC, while the irrigation treatment significantly affected only the growth parameters SW, LW and LA. The allometric indices SLA, LAR and LWC varied independently of irrigation. Nonetheless, lower values of SW, LW, LA and higher values of SLA, LAR and LWC were recorded on plants obtained from the area of Taxiarchis.

Keywords: *Leaf area, Leaf weight, Leaf Assimilation Rate, Specific Leaf Area*.

Introduction

Plants under natural and agricultural conditions are often exposed to various environmental stresses. In Mediterranean basin water deficit is one of the most important factors that curtail plant growth and crop productivity (Zlatev and Lidon, 2012; Cobb *et al.*, 2013). Changes in precipitation patterns, the increase of mean air temperature and the expansion of waterlogging or drought are expected to be intensified in the future due to global climate change (Xoconstle-Cazares *et al.*, 2010; IPCC, 2011). However, for plants grown in dry environments, both a high potential growth rate and efficient use of available water are desirable. The rates of growth and water use are both influenced by the allocation of biomass to the different organs. The allocation pattern that maximizes growth and/or water use efficiency depends on the availability of water. The influence of physiological traits on water use efficiency depends on the balance between the effects on growth and on water use (Zlatev and Lidon, 2012). Plants can adapt to water deficit conditions using physiological, morphological and biochemical mechanisms (Cattivelli *et al.*, 2008; Ghassemi-Golezani *et al.*, 2013). However, the adaptation mechanisms may differ among plant species and genotypes. Breeding of drought-resistant plants can be an advantageous strategy to avoid drought-induced damage to agriculture (Blum, 2011; Zlatev and Lidon, 2012).

Lotus corniculatus L., which grows under a wide range of environmental conditions, is the most important and widely distributed crop from the *Lotus* genus. It is a perennial legume native to the Mediterranean basin and, North Africa and has been widely used in Southern Europe, New Zealand, North and South America (Kirkbride, 1999; Inostroza *et al.*, 2015). It is a drought tolerant species with high nutritive value. However, there is limited published information concerning the physiological mechanisms that cause drought tolerance, as well as, the effects of drought stress on its growth parameters and specifically for populations obtained from different geographical areas (Carter *et al.*, 1997; Acuña *et al.*, 2012; Escaray *et al.*, 2012; Inostroza et al. 2015).

In the present study, *L. corniculatus* seedlings of two different origins (Kilkis and Taxiarchis; northern Greece) were used to evaluate the effect of water deficit on growth characteristics.

Materials and Methods

The experiment was conducted in the farm of the Aristotle University of Thessaloniki, Northern Greece (longitude: 40°31' 91", latitude: 23°59'58"), at an altitude of 6m a.s.l., during the spring of 2013. The climate of the area could be characterized as Mediterranean semiarid with dry summers. The mean annual precipitation is approximately 400mm and the mean annual air temperature is 15.5 °C. Microclimatic conditions in the experimental area, such as air temperature (T), photosynthetic photon flux density (PPFD) and vapor pressure deficit (VPD), were acquired using a microclimatic sensor (Novasima MS1, Novatron Scientific Ltd, Horsham, UK) and are presented in Table 1.

Table 1. Microclimatic conditions of the experimental area during the experimental period of 2013.

Phenological stages	Temperature (°C)	PPFD (μmol $m^{-2}s^{-1}$)	VPD (kPa)
Early vegetative	32.6	872	4.24
Vegetative	27.7	941	2.98
Flowering	24.3	1304	2.02
Fruit formation	30.6	822	2.70

Lotus corniculatus seedlings were sampled from two semi-arid areas of central Macedonia, Greece, namely Kilkis (K, temperature 12.2 ºC; precipitation 585mm; altitude 570m a.s.l.) and Taxiarchis (Tax, temperature 11.1 ºC; precipitation 767mm; altitude 645m a.s.l.) in September and October of 2012 and transplanted in small pots. At the beginning of March 2013, 32 plants from each origin were transplanted –one plant per pot– in larger pots (16cm diameter and 45cm height), filled with medium texture soil collected from the farm. After a two month establishment period, a transparent shelter was placed upon plants in order to prevent rainfall intervention. Two drip irrigation regimes were applied: a) irrigation up to field capacity (FI) and b) limited irrigation (LI) (40% water of that received by FI) in order to maintain water deficit conditions in the soil. The pots were placed in a completely randomized design with four replicates. Measurements were taken on four different dates corresponding to four phenological stages: early vegetative (5/21), vegetative (5/26), flowering (6/2) and start of fruit formation (6/25). In each phenological stage four plants were cut in order to measure the leaf area (LA) and the fresh weight of shoots, stems and leaves.Leaf area was measured using the portable leaf area measurement system Li-3000A (LiCor Lincoln, Nebraska, USA). Then the samples (leaves and stems) were placed in the oven for 48 hours at 70°C to

determine their dry weight. Specific leaf area (leaf area/leaf weight: SLA), leaf area ratio (leaf area/total shoot dry weight: LAR) and leaf water content (leaf fresh weight – leaf dry weight) / leaf fresh weight: LWC) were calculated (Cornelissen *et al.*, 2003).

Analysis of variance (ANOVA) was used to determine the effects of origin, irrigation treatment and plant phenological stage ($P < 0.05$) for all measured parameters (Steel and Torrie, 1980). Independent t-test was used to compare two means. Statistical analysis was performed using the statistical package SPSS (SPSS for Windows, release 22.0; SPSS, Inc., Chicago, USA).

Results and Discussion

The analysis of variance revealed that the plant origin (population) is a significant predictor ($p<0.05$) of SW, LW, LA, SLA, LAR and LWC, while the irrigation treatment significantly affected only the growth parameters SW, LW, LA. The allometric indices SLA, LAR and LWC varied independently of the irrigation regime ($p\geq0.05$). The time of the season (phenological stage) had significant effects ($p<0.05$) on all recorded parameters. The interaction between population and water treatment was not significant ($p\geq0.05$), indicating that there was no difference in the response of the two populations throughout the growing season at the two water regimes. On the other hand, the interaction between population and phenological stage was significant ($p<0.05$) for SW, LW, SLA, and LAR. Both water treatment and plant phenological stage affected stem and leaf dry weights and LA of *L. corniculatus* regardless of the origin. Mean values of dry leaf weight, SW and LA were reduced under water-limited conditions in both origins (Table 2).

Table 2. Average values (± SE) of growth parameters of *Lotus corniculatus* obtained from two origins (Kilkis, Taxiarchis) and subjected to two irrigation treatments in the experimental setting (n=16).

	Kilkis		**Taxiarchis**	
	Irrigation (FI)	Limited Irrigation (LI)	Irrigation (FI)	Limited Irrigation (LI)
Stem weight	11.36±1.55	6.41±0.37	5.59±1.46	2.72±0.58
Leaf weight	7.58±0.81	3.80±0.30	2.45±0.65	1.73±0.47
Leaf area	1664.07±157.43	919.16±122.99	565.53±130.71	359.18±68.35
SLA	231.85±15.84	240.84±31.99	409.22±103.68	304.03±45.11
LAR	95.82±7.84	90.36±12.82	141.28±34.33	133.85±22.27
LWC	0.81±0.010	0.81±0.007	0.85±0.01	0.84±0.02

The dry leaf weight (Fig. 1a) had an increasing trend in the full irrigation treatment while it remained rather stable in the LI treatment in both tested populations. Kilkis population presented the higher values in both irrigation regimes (Table 2, Fig. 1a) compared to Taxiarchis. However, throughout the growing season plants from Kilkis under FI had significantly higher LW compared to plants of the same origin subjected to LI. Only in the early vegetative stage, both populations presented the same LW regardless of the irrigation treatments. One reason may be that the short period of irrigation treatment could not influence

LW at these early stages. In addition, the early phenological stages coincide very often with sufficient rainfall and lower atmospheric temperatures, leading to lower VPD, lower transpirational losses and consequently with less stress in plants; perhaps this was another reason that we did not detect differences in leaf weight between the two irrigation treatments in these stages (Blum, 2011).

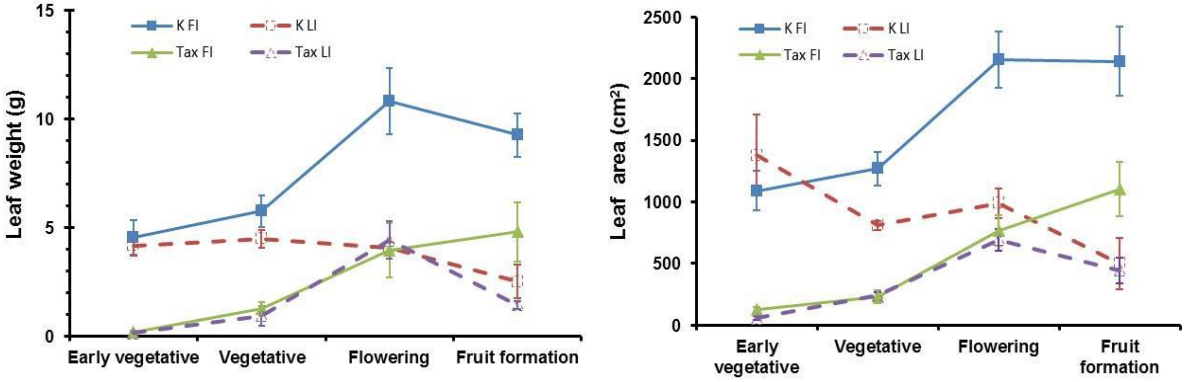

Figure 1. Changes of a) dry leaf weight and b) leaf area at different phenological stages of Kilkis (K) and Taxiarchis (Tax) populations of *Lotus corniculatus*, under full irrigation (FI) and limited irrigation (LI). Values present means ± SE (n=4).

The LA of both populations followed an increasing trend in FI treatment. In the LI treatment though, plants from Kilkis expressed a decreasing while those from Taxiarchis an increasing trend (up to flowering) in LA in response to season progress (Fig 1b). Flowering and fruit formation are recognized as the most drought sensitive plant growth phenological stages. Shrestha et al. (2006) demonstrated that water shortage during the grain filling stage reduced LA in lentils. The decrease of LA may represent a rapid response and a morphological adaptation to reduce water loss and rearrange resources under drought conditions (Zokaee-Khosroshahi *et al.*, 2014). It is well demonstrated that under water deficit conditions plants develop morphological mechanisms, such as decrease in plant dry weight and leaf area to reduce water consumption and continue to sustain life during the dry period of the season (Karatassiou *et al.*, 2014). Not only the pattern of biomass allocation, but also differences in the rates of uptake and loss of CO_2 and H_2O of the different plant organs are expected to contribute to variation in growth and water use efficiency (Zlatev and Lidon, 2012). Under limited irrigation the transpiration rate of Kilkis population was found to be not closely related to stomatal conductance, suggesting a stronger connection to environmental factors such as temperature and VPD changes (Karatassiou et al., 2015) Consequently, in order to understand the influence of limited irrigation on growth and allometric parameters we need to take into consideration the transpiration rate, the assimilation rate and water uptake of the plants.

From the changes of SLA (Table 1, Fig. 2a) during the growing season it becomes obvious that in each origin of *L. corniculatus* plants expressed similar mean values of SLA both under FI and LI. The same trend was recorded for LAR and LWC. Specific leaf area is considered an important attribute of plants to maintain survival in the Mediterranean climate, since it is associated with relative growth rate and plant capacity to use environmental poor resources (Karatassiou et al. 2014). The higher values for SLA were recorded in the first phenological stage for both origins and irrigation treatments. However, plants from Taxiarchis presented higher values of SLA, LAR and LWC (Table 1, Figure 2).

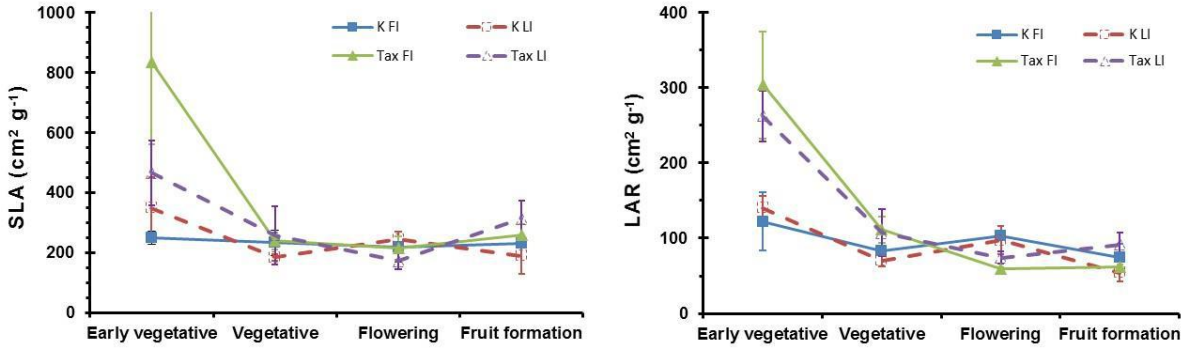

Figure 2. Changes of a) specific Leaf Area (SLA) and b) leaf area ratio (LAR) at different phenological stages of Kilkis (K) and Taxiarchis (Tax) populations of *Lotus corniculatus*, under full irrigation (FI) and limited irrigation (LI). Values present means ± SE (n=4).

The comparable values of SLA, LAR and RWC of both origins of *L. corniculatus* could be probably due to adaptation to poor environments (Li et *al.*, 2005). The above results showed that the two populations of *L. corniculatus* exhibited different responses to water deficit condition as far as growth characteristics are regarded.

Conclusion

Lotus corniculatus populations obtained from two origins of Northern Greece exhibited different growth parameters in response to water stress. The limited irrigation significantly reduced the growth parameters SW, LW and LA but did not affect the allometric ones, SLA, LAR and LWC of both populations.

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Review paper
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DOUBLED HAPLOID LINE PRODUCTION IN DURUM WHEAT: PRESENT STATUS AND FUTURE PROSPECTS

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Abstract

Doubled haploid plant production is a very effective tool in shortening the time needed to develop new varieties. Of the various methods proposed to produce haploid plants anther culture is the most convenient and popular. However, this androgenic response depends on culture medium, on cold or heat pre-treatment, and mainly on the genotype of the donor plants. Although anther culture has been successfully employed in producing haploids in many crop plants, including hexaploid wheat, durum wheat is very recalcitrant to anther-culture, extremely few plants are produced and most of them are albinos. Efforts to produce haploid plants from durum wheat anther culture were attempted by many researchers who evaluated the factors affecting the androgenesis process. They used liquid or solid media, and several medium composition, including the modification of known media, like modified MS, modified W14, C17 etc. The pre-treatment of the anthers with mannitol, PEG, cold or a combined pre-treatment involving mannitol and low temperatures improved green plant regeneration. An alternative approach to obtain durum wheat haploid plants and subsequently doubled haploid lines is the maize technique. Other researchers attempted to cross durum to bread wheat cultivars responding well to anther culture in order to obtain durum wheat haploid plants. A future prospect could be transferring the 1BL.1RS wheat-rye translocation from the bread wheat cultivars to durum wheat and exploit any possible positive effects of the presence of this translocation on durum anther culture.

Introduction

Durum wheat is one widely grown cereal crop in the world and one of the most important ones used for human consumption worldwide. It is cultivated in several European countries including Italy, France, Turkey, Romania, and Ukraine, and in northern Great Plains of Canada and the United States. Genetic improvement to develop new varieties with high yield potential, resistant/tolerant to abiotic and biotic stresses and with acceptable quality is the most viable and environmentally friendly goal in most breeding programs. Isolation of homozygous genotypes through conventional methods requires several cycles of inbreeding and selection, and making this attempt time and money consuming. Doubled haploid plant (DHL) production is a very effective tool in shortening this time, because the homozygosity is achieved in one generation. Of the proposed approaches anther culture is still the most commonly used technique. Although anther culture has been successfully employed in producing doubled-haploids in many crop plants, including hexaploid wheat (*Triticum aestivum* L.) (Kasha and Maluszynski, 2003), this technique has not been proven very successful in durum wheat (Dogramaci-Altuntepe et al., 2001), and extremely few green plants are produced (Cistué et al., 2009). The androgenic response depends on culture medium, on cold or heat pre-treatment, and mainly on the genotype of the donor plants. (Lazaridou et al., 2005). More precisely, in durum wheat the green plant production is greatly genotype dependent. Efforts to produce haploid plants from durum wheat anther culture were attempted by many researchers who evaluated the factors affecting the androgenetic process. They involved the use of liquid or solid media, in several medium composition, including the

modifications of known media, lik e.g. modified MS, modified W14, C17 etc (Saidi et al. 1997). The pre-treatment of the anthers with mannitol (Cistué et al 2006), PEG, cold or a combined pre-treatment involving mannitol and low temperatures (Labbani et al. 2007) improved green plant regeneration. An alternative approach to obtain durum wheat haploid plants and subsequently doubled haploid lines is the maize technique which is less genotype dependent (Jauhar et al. 2009). Finally, Tersi et al. (2005) attempted to improve anther culture response of durum wheat by transferring B genome chromosomes by crossing durum to bread wheat cultivars responding well to anther culture.

In this review, the various efforts to obtain durum wheat DHLs through anther culture, wheat x maize wide crosses, and other alternative methods, as well as the future prospects, are summarized.

DOUBLED HAPLOIDS THROUGH ANTHER AND MICROSPORE CULTURE:

Although the first success in regeneration of bread wheat (*Triticum aestivun*) plants through anther culture was achieved in the early 70's (Ouyang et al., 1973), little progress has been reported in durum wheat. This is because durum wheat is a very recalcitrant to anther-culture crop, few embryos are formed (Foroughi –Wehr and Zeller 1990) and most of the pants produced are albinos (J'Aiti et al., 2000; Labbani et al., 2005; Cistué et al., 2006, 2009). During anther culture, microspores are induced to produce haploid plantlets after going through various developmental stages. This process of haploid production is influenced by physiological factors such as growth conditions of donor plants, developmental stage of microspores, types of pre-treatments and composition of the induction and regeneration media (Liu et al., 2002; Zheng et al., 2003; Cistué et al., 2009; Jauhar, 2003a). Different investigations have been carried out in order to evaluate the effect of these factors in anther culture response and to develop a protocol to regenerate green haploid plantlets from durum wheat anther culture. Several liquid or solid media, and several medium composition and modifications have been applied. Cherkaoui et al. (1997) used three liquid media: BPTG (Chuang et al, 1978), C17 (Wang and Chen,1986), BAC -1 (Marsolais and Masha, 1985), and tested nine Moroccan genotypes for their anther culture response. They reported green plant regeneration from five cultivars and five doubled haploid green plants were produced from BAC-1 medium. Saidi et al. (1997) who also evaluated Moroccan durum wheat genotypes found that pretreating of spikes at 3°C for eight days improved the androgenetic capacity. They used several culture media and found that the C17 medium was consistently the best for embryo induction. However, only two green plants from two cultivars were produced. Jauhar (2003a) reported that the BAD-1 medium (Trottier et al., 1993), is the most suitable induction medium for anther culture of durum wheat. The same medium has been used as a differentiation medium. Among the green regenerants several disomic (2n=28) polyploid and aneuploid plants were recorded. Lazaridou (unpublished data) observed that the use of induction medium W14 (Ouyang et al., 1989), supplemented with 5.0 mg/l 2,4-D + 0.5 mg/l BAP + 20.0 mg/l Ethephon + 50.0 mg/l L-cysteine + 500.0 mg/l myo-inositol (Kiviharju et al., 2005) , and pre-treatment consisting in a combined mannitol 0.7 M and cold for 7 days had a strong effect on the number of embryos produced after durum wheat anther culture. The second approach, microspore culture, is considered as more appropriate for durum DHL production than anther culture. Microspores floating in a medium with high viscosity are developed into embryos with normal morphology (Cistué et al. 2004). Jauhar (2003a) reported that four different induction media have the ability to produce calli from durum wheat microspores e.g.: BAC-1 (Marsolais and Masha, 1985), BAD-1, BAD-3 (Trottier et al., 1993), and M-42 (Kao et al., 1991). Labbani et al. (2007) reported the positive effect on callus formation of liquid CHB3 medium (Chu et al. 1990) after a proper modification. Durum wheat microspore culture was practically ineffective until ovaries were added into the

induction medium. Co culture with ovaries has been tested and found to be beneficial in durum wheat (Cistué et al 2006). Anthers of five cultivars and 20 F_1 crosses were pre-treated in 0.7 M mannitol, and microspores were plated on a C17 induction medium with ovary co-culture. Green plants were regenerated from all genotypes used and most of them were spontaneously doubled haploids. Some haploids and a very few polyploids plants were obtained. The production of many aneuploid plants is another problem in anther culture of durum wheat. Doğramaci-Altuntepe et al. (2001) studied the anther culture response of 10 durum wheat cultivars using three different growth conditions and four culture media and observed several chromosomal abnormalities in the plants produced. Cistué et al (2009**)** using C17 induction medium with ovary co-culture and a supplement of glutathione plus glutamine, 300 g/l Ficoll Type-400, managed to maximize production of green plants per spike from three commercial cultivars and ten F_1 crosses. Many green plants were obtained and no aneuploid plants were obtained.

Osmolality plays an important role in the inward and outward movement of water from plant cells. Depletion of water by osmotic stress is an important signal, or pretreatment, that triggers microspore embryogenesis and directs proper embryo development in various plant species (Wojnarowiez et al. 2004). Various stress pretreatments were applied including cold (Labbani et al., 2005), and osmotic shock (Cistué et al., 2006; Labbani et al., 2007). It was demonstrated by Labbani et al. (2005) that a cold pre-treatment of the microspores at 4° C for five weeks could be promising for green regeneration in durum wheat. Cistué et al. (2006) reported that anthers pre-treated in 0.7M mannitol for 5 days improved green plants regeneration. Labbani et al. (2007) showed that a pre-treatment consisting in a combined mannitol 0.3 M and cold for 7 days had a strong effect on the number of embryos produced and the regeneration of green plants. Ayed et al. (2010), in order to further improve the rate of haploid production through durum wheat microspores culture, have compared different types of stress conditions, including a combined pre-treatment involving mannitol or PEG and low temperatures. They reported that cold treatment for five weeks (4°C) was the most efficient, and that the involvement of PEG4000 1% for 10 days seemed to be a promising pretreatment.

WIDE CROSSES - MAIZE TECHNIQUE

Wide crosses have been utilized for the production of haploids for crop improvement and genetic studies (Baum et al. 1992). Bread wheat DHs are produced by various intergeneric crosses with maize (Inagaki and Tahir 1990; Laurie and Reymondie 1991), and *Hordeum bulbosum* (Barclay 1975). Although the *H. bulbosum* mediated haploid production has been accomplished in bread wheat and has a widespread use in doubled haploidy programs in barley, this method has not been effective in durum wheat (O'Donoughue and Bennett 1994). Crossability of wheat × *H. bulbosum* depends on the wheat allelic composition for the Kr genes responsible for the incompatibility between these two species (Sitch and Snape 1987). The production of haploids by hybridization with maize has been reported in hexaploid wheat (Inagaki 2003, Ushiyama et al. 2007, Xynias et al. 2014) and durum wheat (Almouslem et al. 1998, Jauhar 2003b).

The effect of individual durum wheat (*Triticum turgidum* L.) chromosomes on crossability with maize (*Zea mays* L.) was investigated by Doğramaci-Altuntepe and Jauhar (2001). Doğramaci-Altuntepe and Jauhar (2001) used 14 'Langdon' (LDN) D-genome disomic substitution lines, a LDN Ph mutant (Ph1b ph1b), and normal 'Langdon' line, which were pollinated with maize pollen. Haploid embryos were obtained from all lines but only 14 of the embryos germinated and developed into healthy plants. Different substitution lines exhibited varying degrees of success. These results indicate that the substitution of 5D for 5B confers on durum wheat a greater ability to produce haploids.

Maize - mediated haploidy induction is the method that has helped to produce haploids from several durum cultivars and genetic stocks (Almouslem et al. 1998). Generally in the case of durum wheat x maize zygotes , the maize chromosomes are completely eliminated after the third mitotic. Very rarely however a maize chromosome may be retained in the resulting durum haploid plantlet. Jauhar et al. (2009) observed a durum haploid plant with 15 chromosomes which presumably included a maize chromosome.

An alternative approach to overcome the recalcitrancy of durum wheat (*Triticum turgidum* var. *durum*) to anther culture, was attempted by transferring the responsible genes form bread wheat B-genome to the respective of durum wheat (Tersi et al. 2005). Three durum wheat cultivars were crossed to two bread wheat (*Triticum aestivum* L. em Thell) cultivars and anthers from the resulting F_1 plants and their original cultivars were cultured on potato-2 and W_{14} media. No green plants were produced from the parental durum wheat cultivars. In contrast, green plants were produced from the F_1 plants and the cytogenetic evaluation revealed that they all carried D-genome chromosomes.

Conclusion and perspectives

Haploid plants and haploid–derived homozygous lines are useful in plant breeding programs. Anther culture is a very useful technique for the production of doubled haploids, and thus to the production of DHs through androgenesis, is a proven method to obtain homozygous individuals in a single step. Durum wheat is very recalcitrant to anther-culture, with extremely few plants produced and most of them are albinos. On the other hand maize–induced chromosome elimination approach offers an alternative way in durum haploid plant production. However, the improvement in microspore culture could change this conclusion. A future prospect could be the formation of an improved protocol for anther or microspore culture of durum wheat that involves both cold and osmotic shock. Another prospective could be transferring the 1BL.1RS wheat-rye translocation from the bread wheat cultivars to durum wheat, because it is well known that the presence of 1BL/1RS translocation in bread wheat cultivars improves the embryoid production and green plant regeneration from anther culture. For this, transferring of the 1BL.1RS wheat-rye translocation could improve the anther culture response of durum wheat and consequently any other possible positive effects of the presence of this translocation on durum wheat could be exploited.

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Original scientific paper
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INFLUENCE OF DIFFERENT STRAINS OF AGROBACTERIUM RHIZOGENES AND EXPLANTS AGE ON INDUCTION OF HAIRY ROOTS AND PRODUCTION OF HYOSCYAMINE AND SCOPOLAMINE IN HYOSCYAMUS RETICULATUS

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Abstract

Different *Agrobacterium rhizogenes* strains and three various explants age (one week old, two weeks old, four weeks old leaf) were tested to investigate the ability for transformation and production of secondary metabolites in *Hyoscyamus reticulatus*. All the strains of *A. rhizogenes* (A7, 15834, A13 and D7) used in this study produced hairy roots. Furthermore, effects of explants type (Cotyledon, Hypocotyl, two weeks old leaf, two weeks old internode, four weeks old leaf, and four weeks old internode), effects of two inoculation methods (immersion and injection) on hairy roots induction in cotyledon explants, different culture media (MS, 1/2MS, 1/4MS and B5) were investigated. Hyoscyamine and scopolamine production as a main product in *H. reticulatus* were measured by GC/MS. The results show that, induction of hairy roots in *H. reticulatus* was effected by strain and explant type. So, after 19 days, A7 strain and cotyledon explants were the best explants for induction of hairy root in *Hyoscyamus reticulatus*. ANOVA results showed that, the growth of hairy roots was significantly affected by medium type and the highest fresh weight produced in MS and B5 medium. Hyoscyamine (2/6 fold) and scopolamine (7/56 fold) content were increased significantly in Transgenic roots compared with non transgenic roots.

Key words*: Agrobacterium rhizogenes*, Bacteria strain, Hairy root, *Hyoscyamus reticulatus*, stem internode.

Introduction

Hyoscyamus reticulatus is an important medicinal plant, distributed in China, Afghanistan, India, Japan, Korea, South West Asia, North Africa and throughout Europe (Sajeli *et al*., 2006). *H. reticulatus* contains two distinguished tropane alkaloids, Hyoscyamine and scopolamine and both the compounds possess potential acute or chronic toxicity (Vike *et al*., 2008). It has been reported that tropane alkaloids are mostly synthesized in the young root cells and then transported to the aerial parts (Hashimoto *et al*., 1991). Nowadays hairy root cultures from plants are getting considerable attention because of their genetic and biochemical stability, rapid growth rate and ability to synthesize secondary products at levels comparable to the original plants (Srivatava and Srivatava, 2007). In addition, this type of culture is known to produce a spectrum of secondary metabolites that are not present in the parent plant (Reerasham, 2004). During the past two decades considerable efforts have been made to develop an economically feasible *in vitro* production of these compounds. Therefore, in this study the effect of influencing factor including, various explants age, different strains of *A. rhizogenes*, explants type, two inoculation methods and different culture media were investigated.

Materials and methods

Plant Material

Dormancy of seeds was broken by immersing them in 100 ppm GA_3 solution for 24 h. Seeds of *H. reticulatus* were surface-sterilized, Then they were cultured on semi solid hormone-free MS (Murashige and Skoog, 1962) medium and incubated at 25±2°C for a photoperiod of 16 h light.

Bacterial strain

Mono clone of four *A. rhizogenes* strains (A7, 15834, A13 and D7) was grown for 24 h in liquid LB medium with rifampicin 0.50 g/L antibiotic (Sigma Chemical Co.) At 28^{o}C with shaking (180 rpm) in liquid LB medium, to mid-log phase (OD600 = 0.5).

Establishment of hairy root cultures

Leaves of *H. reticulatus* obtained from *in vitro* grown plants at three old types explants (one week old, two weeks old, four weeks old leaf). Excised leaves were dipped into four *A. rhizogenes* strains (A7, 15834, A13 and D7) cultures in liquid inoculation medium for 1 min, blotted dry on sterile filter paper, and incubated in the dark at 25°C on agar-solidified MS medium. After 2 days of co-cultivation, the explant tissues were transferred to a hormone-free medium containing MS salts and vitamins, 30 g/l sucrose, 500 mg/l cefotaxime, and 8 g/l agar. Numerous hairy roots were observed emerging from the wound sites of explants within 10 days.

The hairy roots were separated from the explant tissue and subcultured in the dark at 28°C on agar-solidified MS medium.

Inoculation methods

Leaves obtained from cotyledon explant *in vitro* grown plants were infected by bacterial strain A7 and the effects of two inoculation methods (immersion and injection) were tested. In immersion method explants were pricked with sterile needle dipped in bacterial suspension for 1 min and in injection method bacterial suspension were injected with excel (0/5 mM) to cotyledon explants.

Effect of different cultivation medium

Four commonly used standard plant cell cultivation media viz. MS, B5, 1/2MS, and 1/4MS (Gamborg *et al*., 1968) were tested for their effect on growth and antioxidant activity in hairy root cultures of *H. reticulatus*. The hairy root culture from selected root line was initiated by transferring fresh hairy roots equivalent to 1 g into 250 ml Erlenmeyer flask containing 30 ml of liquid B5 medium. The cultures were incubated on a incubator shaker at 1000 rpm and 28°C. The flasks were harvested after 21 days to analyze DW and antioxidant activity content.

PCR analysis of hairy roots

Genomic DNA was extracted using CTAB method (Khan *et al*., 2007) from each of the hairy root lines as well as from control roots. PCR primers specific for the amplification of the 780 bp fragment of the *rol* B gene were used.

Extraction of alkaloids and GC/MS analysis of hyoscyamine and scopolamine

One gram of dried and powdered roots of *H. reticulatus* was extracted with methanol in a Soxhlet apparatus for 130 min. Then 1 μL of extract was directly injected into the GC/MS. The results were obtained as the mean of 3 separate injections. Antioxidant activity analyses were performed by (Chiuo *et al*., 2007) method.

Results and discussion

Effect of different *A. rhizogenes* strains on hairy root induction

The resulted indicated that interaction effect *A. rhizogenes* strains and explants age were significantly effect on time period requirement for hairy root induction and transformation frequency. All A7, 15834, A13 and D7 strains of *A. rhizogenes* used in this study were able to produce hairy roots at the explants infection site (Figure 1). The highest infection frequency (76/22%) found in A7 strain and one week old explants. The lowest infection frequency (14%) was observed in D7 strain and four weeks old explants. Efficiency of transformation is known to differ with different bacterial strains (Dogan *et al*., 2011). Different strains of *A. rhizogenes*

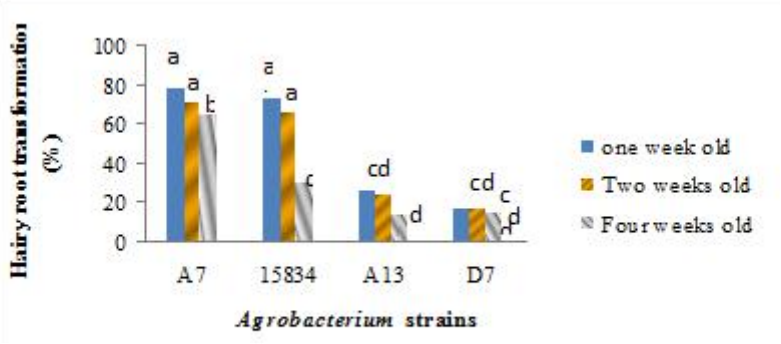

Figure 1. Effect of different strains of *A. rhizogenes* on hairy root induction

Produce different types of opine (Isogai *et al*., 1990). Soil-borne pathogens of genus *Agrobacterium* are able to transfer part of their DNA, the T-DNA carried on a large plasmid, to the genome of a host plant cell. Furthermore, explant age is also an important factor that affects on hairy root induction and growth (Vergauwe *et al*., 1998).

Effect of explants type

Among the different explants tested (Cotyledon, Hypocotyl, two weeks old leaf, two weeks old internode, four weeks old leaf, four weeks old internode) cotyledon explants induced hairy roots at greater percentage (77/66%) whereas the four weeks old internodes segments were less than other explants comparatively (Figure 2). Juvenility and nature of explant influence the *Agrobacterium* mediated transformation process (Trypsteen *et al*., 1991). Nin *et al* (1997) have reported that specificity of *Agrobacterium* transformation is closely connected with the age and hormonal balance of the host tissue. It is quite evident that different explants vary in their wound response, that is, produce the number of competent cells for transformation.

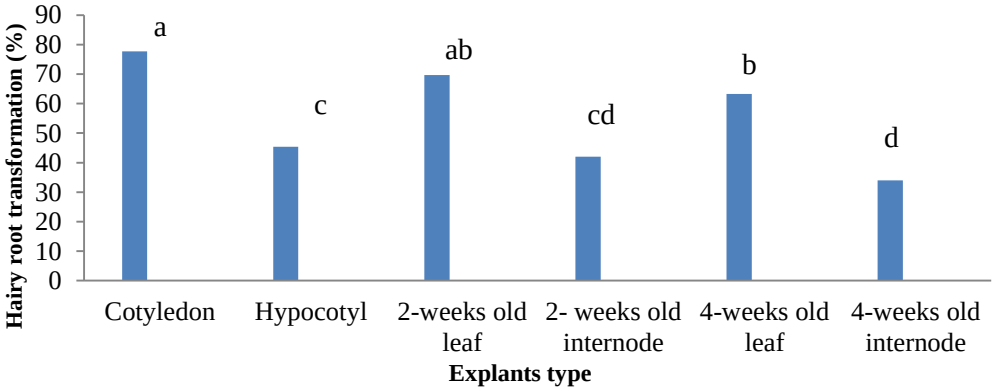

Figure 2. Effects of explants type on transformation frequency.

Effect of different *A. rhizogenes* strains on time period for hairy root induction

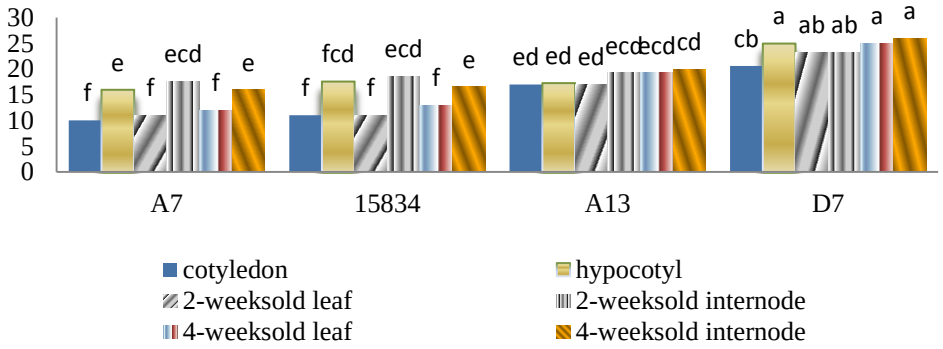

Figure 3. Effect of different strains of *Agrobacterium rhizogenes* and explants type on hairy root appearance

Hairy roots grow rapidly, show plagiotropic growth, and are highly branched on phytohormone free medium. The resulted that hairy roots were induced 10 days after infection with A7 strain and cotyledon explants (Figure 3). The growth rates of transformed roots are known to vary greatly between species, but differences are also observed between different root clones of the same species (Mano *et al*., 1989).

Effect of different cultivation medium

Medium types (MS and B5) and strength (full strength and half strength) displayed significant effect on both fresh and dry weight of *H. reticulatus* hairy roots. B5 and MS medium detected as the best medium for the growth of hairy roots. Fresh and dry weight of hairy roots grown in B5 medium were significantly higher (0.32 gr) than other medium after 21 days (Figure 5).
Media manipulations have been reported to aid in the release of metabolites. Betacyanin release from hairy roots of *Beta vulgaris* was achieved by oxygen starvation (Boitel *et al*., 1996). As the medium strength decreased to half strength, solasodine level in *Solanum mauritianum* hairy roots was lowered but ajmaline and ajmalicine levels in *Pauvolfia micrantha* were increased (Sudha *et al*., 2003).

Molecular identification and quantification of hyoscyamine and scopolamine

PCR results with specific primers showed that all the hairy root lines contained *rolB* gene which was a part of Ri plasmid T-DNA in *A. rhizogenes* (Figure 7).

GC/MS analysis

Hyoscyamine (2/6 fold) and scopolamine (7/56 fold) content were increased significantly in Transgenic roots compared with non transgenic roots (Table 3). Transgenic roots can increase the activation of enzymatic pathways that contribute to the production of secondary metabolites, hence, scopolamine increase in hairy roots, can be due to increase in Hyoscyamine-6-hydroxylase enzyme activity that convert hyoscyamine to scopolamine (Dehgan *et al*., 2012) These results suggest that hairy roots can be used as a new powerful pathway to increase the yield of secondary metabolites in plant biotechnology.

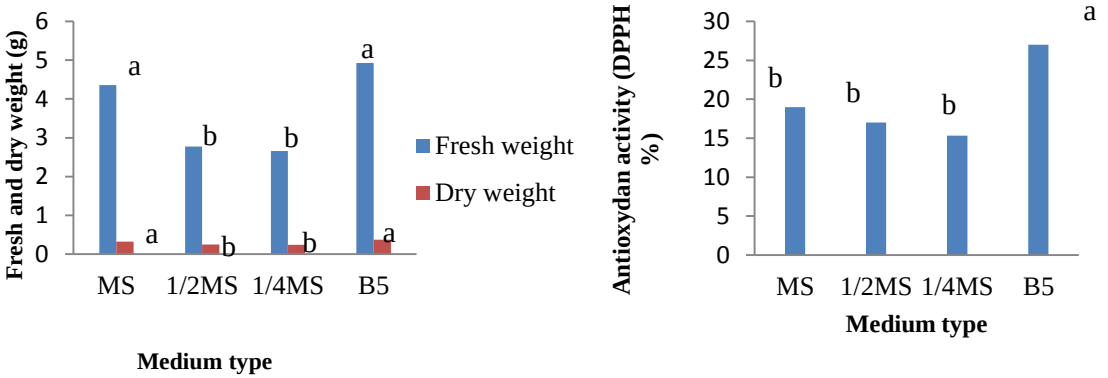

Figure 5. Influence of medium strength and types, on *H. reticulatus* hairy root biomass and antioxidant activity

Table 3. GC/MS assess the roots *H. reticulatus* for the production hyoscyamine and scopolamine

	Tropan alkaloid	
	Hyoscyamine (%)	Scopolamine (%)
Non transgenic root	0.18	0.37
transgenic root	0.48	2.8

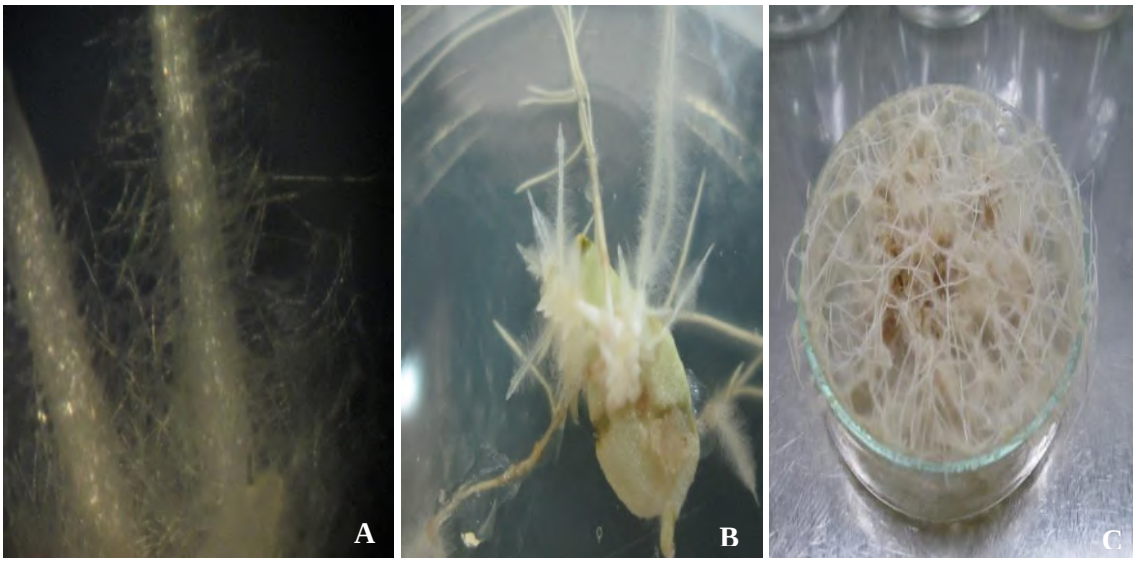

Figure 4. A7 induced hairy roots incotyledon explants of *H.reticulatus.*A) Hairy root appearance B) Arrows indicate the hairy root induced on different parts of the explant C) Hairy roots of *H.reticulatus* growing on hormone-free B5 medium.

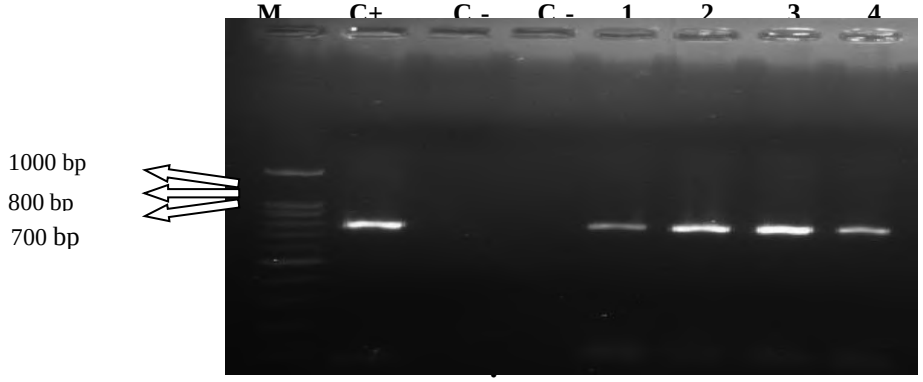

Figure 7. Agarose Gel electrophoresis of PCR products. The *rol*B gene size is 780 bp. M: marker DNA (1 Kb); C+: *A. rhizogenes* plasmid DNA. C_1- and C_2- : control root. lane 1 to 4: *H.reticulatus* hairy roots.

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Original scientific paper
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THE EFFECT OF FARM SLOPE AND APPLICATION OF DIFFERENT FERTILIZER SOURCES ON WHEAT YIELD

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Abstract

This experiment was done as split plot based on randomized complete block design with three replications in Piranshahr-Iran, during growing season of 2011- 2012, to determine the effect of farm slopes on yield and some agronomic traits of wheat under different fertilizer sources. Treatments consisted of farm slope at four levels: (0, 3, 6 and 10 percent) as the main plots and combination of different fertilizer at five levels: [control, application of biofertiliozer (Nitroxin), manure, chemicals (N and P] and integration of biological, manure and chemical as sub plots. Results showed that with rising of farm slope, a significant decrease revealed in the spikelet per spike, number of grains per spike, 1000-grain weight, wheat straw yield, biomass and grain yield. In fact, with increasing the farm slope, the yield components decreased which lead to 52 and 38 percent reduction in grain yield and total biomass of plants located on a slope of 10 percent than the control plants. The applications of integrated fertilizer treatments had the most effect on morphological traits and yield components of wheat that increased 12.58 and 13.58 percent the grain yield and total biomass compared to the control plants. Generally, integrated fertilizer treatments that reduced half of chemical fertilizers application, recommended to development of ecological agriculture in the wheat fields.

Keywords: *Biofertilizer, Integrated nutrition, Manure, Morphological traits, Yield component*

Introduction

The most important issue that is critical to the food security of the world population is food supply such as wheat that increasing its production has been emphasized. Increasing wheat production through the development of acreage due to constraints of resources are impossible, but the only reasonable approach is trying to improve the yield per area.

The crop yield affected by various factors, which is one of the most important, is farm topography (Kravchenko and Bullock, 2000). Land topography effected soil physicochemical characteristics of the soil via erosion (Ebeid *et al.*, 1995), a change in soil organic matter (Kravchenko and Bullock, 2000), the amount of water available in the soil (Verity and Anderson, 1990) and the nutrients. The characteristic of topography in agriculture is important factor in the performance of agricultural products due to a direct effect on the microclimate, soil properties such as temperature and soil moisture (Godwin and Miller, 2003). Farm slope is one of the most important indicators that has a major impact on the nutrient balance and hence reduce soil fertility.

Quantitative and qualitative characteristics of crops are affected by nutrient (Tanaka *et al*., 1997). Supply of nutrients such as nitrogen through the use of chemical fertilizers in the farm with slope, which are prone to washing, is one of the main reasons for pollution of the water cycle in ecosystem. So, replacing it with organic and biological fertilizers plays an important role in the environmental health (Chandrasekar *et al*., 2005).

integrated use of chemical fertilizers and organic fertilizers such as manure and biological fertilizers that called integrated management of nutrient, improve soil fertility and biological

activity of the soil physical properties (Berecz *et al*., 2005) and thus may be more effective than using either of them alone (Montemurro, 2009).
As regards to the most of farm under wheat cultivation in the world has slope, so understanding how the reaction of wheat growth characteristics with changes in the slope of the land is important.
The aim of this study was to evaluate the response of wheat in relation to the slope and use of different sources of fertilizer.

Materials and methods

This field experiments were performed in the research field of Piranshahr, Iran (36°67′ N, 45°21′ E, altitude 1460 m, West Azarbaijan Province) during growing season of 2011- 2012. Some physicochemical characteristics of soil, based on farm slope treatment were given in Table 1.
Treatments were farm slope at four levels: (zero, 3, 6 and 10 %) as main plots and combination of different fertilizer at five levels: (control, application of biofertiliozer (Nitroxin), manure, chemical (N and P) and Integrated (biological +manure +chemical) were considered as sub plots.

There was farm slope as naturally and farm slope determined using the leveling model DSZ-2. The chemical fertilizer were used according to soil physicochemical properties (Table 1) at 100, 120, 140 and 160 kg/ha of urea and 80, 100, 120 and 140 kg/ha triple super phosphate, respectively in plots with a slope of zero, 3, 6 and 10%. Half of urea fertilizer was used at planting and the rest used at stem elongation. In the manure treatment, 10, 15, 20 and 25 t/ha cow manure were used, for farm slopes from zero to 10 percent, respectively. Nitroxin that include *Azotobacter chorococum, Azospirillum lipoferoum* was used as bio-fertilizer treatment.
In the plots with integrated fertilizer treatment, a combination half of the amounts mentioned of chemical fertilizers, manure and biofertilize were used respectively, in plots with a slope of zero, 3, 6 and 10 percent. Totally, there were 20 experimental treatments, 60 experimental plots, and each plot set as ten rows (30 cm inter) with 400 cm long.

Seeds of *Triticum aestivum* L. *cv*. Zarin were sown in December 2011. Environmental conditions of the experimental site, including the highest and lowest temperature, humidity and rainfall, during the study are shown in Table 2.
At plant maturity, the number of spikelets per spike, grains per spike, grain weight, the weight of straw, biomass and grain yield were measured. All measurements in each plot, after the elimination of margins (two rows of both sides and 50 cm from the beginning and the end of rows), was done at the middle row randomly. Analysis of the data was done using SAS software. For mean comparison SNK method was used at the level of five percent.

Table 1. Soil physicochemical properties of field experiment conditions in different slopes.

Slope (%)	Soil tex.	EC (dS/m)	pH	Organic C (%)	Total N (%)	P (mg/kg)	K (mg/kg)
0	Clay-loam	0.55	7.1	0.7	0.08	9.5	392
3	loam	0.51	7	0.62	0.071	7.2	320
6	Sandy-loam	0.45	7	0.51	0.067	6.1	275
10	Sandy-loam	0.4	7.2	0.41	0.051	5	245

Table 2. Some metrological parameters of the experimental site (Piranshahr) during the study period.

	2012				2011				
	Jul	Jun	May	Apr	Mar	Feb	Jan	Dec	Nov
Minimum temp. (◦C)	18.1	15.2	10.5	5.2	-3.2	-2.9	-1.6	-3.7	2.2
Maximum temp. (◦C)	31	27.3	23	15.3	4.3	3.8	6.3	5.1	9.8
Relative humidity (%)	39	38	50	52	65	71	71	71	69
Rain (mm)	7.7	3.4	5.7	92.5	98.8	133.4	47.6	17.1	127.3

Results and discussion

Results showed that number of spikelet’s per spike, grains per spike, 1000-grain weight, straw yield, biomass and grain yield of wheat plants affected by treatments and have significant differences compared to mean control value (Table 3).

Table 3. Variance analysis of various traits of wheat under different fertilizer systems and farm slope

Source of Variation	df	Mean square					
		Spikelet’s in spike	Grains in spike	1000-grain weigh	Straw yield	Biomass	grain yield
Block	2	2.01	42.83	0.36	0.48	3.8	1.77
Slope	3	71.47^{**}	1662^{**}	76.48^{**}	28.07^{**}	146^{**}	46.7^{**}
Main plot error	6	1.24	33.37	1.26	0.6	3.21	2.09
Fertilizer	4	14.1^{**}	362.2^{**}	11.96^{**}	5.36^{**}	11.87^{**}	1.37^{**}
Slope*Fertilizer	12	0.38^{ns}	15.73^{ns}	0.54^{ns}	0.27^{ns}	0.26^{ns}	0.13^{ns}
Error	32	0.76	18.42	0.85	0.27	0.5	0.23
C.V (%)		5.41	10.47	2.05	5.47	4.6	8.44

ns: Non significant, * and **: significant at 0.05 and 0.01 probability level, respectively.

By increasing the slope of the farm, reducing the number of spikelets per spike was observed. So, the highest number of spikelet’s per spike (18.5) and the lowest number (13.5) of it, were observed in plants located in zero and 10% farm slope, respectively (Table 4). In the farm that has high slope, washing minerals and lower soil water holding capacity, leading to a reduction in the growth and development of plants (Kravchenko and Bullock, 2000).

In fertilizer treatments, also the highest number of spikelets per spike (17.6) was observed in the plants that treated with integrated fertilizer that show the role of nutrients diversity in growth and development of plants (Table 5).

Table 4. Mean comparison of wheat traits at different farm slope

Farm slope (%)	Spikelet’s in spike	Grains no. in spike	1000-grain weigh (g)	Straw yield (t/ha)	Biomass (t/ha)	grain yield (t/ha)
0	18.5^{a}	53.15^{a}	47.2^{a}	11.14^{a}	18.8^{a}	7.67^{a}
3	17.1^{b}	45.31^{b}	46.63^{b}	9.88^{b}	16.4^{b}	6.55^{a}
6	15.3^{c}	36.64^{c}	44^{c}	9.13^{c}	14^{c}	4.88^{b}
10	13.5^{d}	28.78^{d}	42.4^{d}	7.88^{d}	11.5^{d}	3.68^{b}

Similar letters in each column indicated no significant difference at 5 % level of probability.

The maximum number of grains per spike (53.1 grain) was obtained in plants located on the slope of zero and the lowest number of grains (28.8 seed) belongs to the wheat plants in 10% of farm slope. As well as, the highest number of grains per spike (48.6) observed in use of integrated fertilizer treatment (Table 5). In fact, the number of grains per spike in the wheat plants that treated with chemical, manure and biofertilizer treatments showed no statistically differences with each other and were in one group (Table 5). Chemical fertilizers and

livestock manure treatment in combination with biological have a rich source of macro and micro nutrients, and use of them in the soil with regards to the gradual release of nutrients, stimulates microbial activity in soil leading to increases plant growth conditions (Ahmad *et al.*, 2007).

Table 5. Mean comparison of wheat traits under different fertilizer treatment

Fertilize treatment	Spikelet's in spike	Grains no. in spike	1000-grain weigh (g)	Straw yield (t/ha)	Biomass (t/ha)	grain yield (t/ha)
Chemical	16.9[a]	44.8[b]	45.8[a]	10.13[a]	16.1[a]	5.94[a]
Bio fertilizer	15.7[b]	37.5[c]	44.5[bc]	9.06[ab]	14.4[c]	5.36[b]
Manure	15.7[b]	38.53[c]	44.7[b]	9.49[a]	15.1[b]	5.67[ab]
Integrated	17.6[a]	48.58[a]	46.2[a]	10.2[a]	16.2[a]	6.12[a]
No fertilizer	14.8[b]	35.39[c]	43.8[c]	8.66[c]	14[c]	5.35[b]

Similar letters in each column indicated no significant difference at 5 % level of probability.

1000-grain weigh of wheat plants that growth from zero to 10% of farm slope, shows a significant decline (Table 4). Also the highest 1000-grain weigh (46.2 g) was obtained in wheat treated with integrated fertilizer, which increased 5.19 % the 1000-grain weigh of wheat in comparison with control plants (Table 5).

Wheat straw yield was also significantly affected by the experimental treatments (Table 3). The lowest (7.88 t/ha) and the highest (11.14 t/ha) of wheat straw yield was belong to the plants located in 10 and zero percent of farm slope, respectively (Table 4). Increase of farm slope from zero to 10% cause to decrease 29% by weight of straw (Table 4). Results showed that the maximum of wheat straw yield was obtained in wheat plants that treated with integrated fertilizer treatment (Table 5).

The results of the mean comparison showed that the highest biomass of wheat (18.8 t/ha) was belong to the plants at zero slope of the farm and the lowest value (11.5 t/ha) was related to the 10% of farm slope (Table 4). In the fertilizer treatment the highest of wheat biomass was obtained in plants that treated with integrated and chemical fertilizer (Table 5). In fact combined use of bio-fertilizers with manure and chemical fertilizer cause to improve the efficiency of fertilizer by leading to increase of morphological traits and yield components of wheat and thus increase plant biomass (Table 5).

The highest (7.67 t/ha) lowest (3.68 t/ha) of wheat yield were observed in plants at the zero and 10 % of farm slope, respectively (table 4). Therefore, increasing slope from zero to 10% cause to decreasing 52% of the wheat yield (Table 4). The microclimate conditions and physiochemical characteristics of soil in different farm slopes cause increasing wheat yield from zero to 10% of farm slope (Godwin and Miller, 2003).

Wheat plants that treated with integrated fertilizer had the highest seed yield (6.12 t/ ha) and the lowest of wheat yield (5.35 t/ha) obtained from control plants (Table 5). Integrated fertilizer system that includes component of chemical fertilizer, manure and biofertilizer, through positive effects on biochemical reactions, photosynthesis, increasing during the plant growth, shoot dry matter accumulation and yield components cause to increasing the wheat yield (Pilbeam *et al.*, 1997).

Conclusion

The results obtained from this study showed that with increasing in farm slope (from zero to 10%), the wheat yield components such as number of spikelet's, number of grains per spike and 1000-grain weight decreased that leads to a reduction of 52, 38 and 29 percent of grain yield, biomass and straw yield, respectively. Also the highest value of yield and yield components of wheat were obtained in wheat plants that treated with integrated fertilizer system. Thus, the integrated fertilizer treatments with reduced use of chemical fertilizer, recommended to the development of ecological and sustainable agriculture in the wheat fields.

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EFFECT OF ACHENES PRE-HYDRATION IN SOLUTION OF SILICA NANOPARTICLES ON EARLY GROWTH PERFORMANCE OF SUNFLOWER (Helianthus annuus L.)

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Abstract

Seed germination and early seedling growth are the most important stages of plant development. Fast seed germination, stand establishment and uniform field emergence are critical factors to crop production and are essential to achieve optimum performance. Controlled imbibition of seed followed by dehydration could be applied for reducing the time from sowing to seedling establishment and improving germination rate. In present study the effect of pre-germination hydration of sunflower seeds in solutions containing different concentrations of nano-silica (0, 0.2, 0.4, 0.6, 0.8, 1 and 1.2 mM for 8 h) on germination characteristics were investigated. Seed pre- sowing treatment in low concentration of silica nanoparticles (0.2 and 0.4 mM) reduced days to 50% germination and mean germination time and improved germination energy, root length, mean daily germination, germination rate, seedling vigor index, promptness index and final germination percentage. The results of the present experiment agreed with the conclusion that pre-sowing seed treatments with low concentration of nanosilica had favourable effect on sunflower seed germination and seedling early growth. The findings are noteworthy in terms of utilization of suitable concentration nanoparticles as an efficient seed vigour enhancement treatment to improve seedling growth under environmental conditions.

Keywords: *nanosized, silicon oxide, seed priming, germination rate, seedling vigor*

Introduction

Nowadays it has become important to increase crop production to feed the growing world population. To meet this increasing demand, researchers are trying to develop an efficient and ecofriendly production technology based on the innovative techniques to increase seedling vigour and plant establishment through physical seed treatments. Seed germination is an important phenomenon in modern agriculture because it is a thread of life of plants that guarantee its survival.

Poor crop establishment was identified as a major constraint on rainfed crop production (Harris et al., 1999). In this context, it seems that seed priming as one of the seed invigoration strategies would improve seed germination, plant growth, and crop yield. Pre-sowing seed treatments or seed priming performed by various approaches and methods enhances pre- and post-germination activities. Priming is adjusting the hydration level within seeds to permit seedlings to emerge more rapidly and to facilitate the uniform seedling stand (Basra et al., 2006). The main principle of pre-sowing seed treatments is controlled hydration of seed to a point where germination processes are started but not fulfilled. Most priming treatments involve imbibing seed with constrained amounts of water to allow adequate hydration and progress of biochemical processes but hindering the protuberance of the radicle (Ashraf and Foolad, 2005). In respect of the pre-sowing seed treatments effect Wahid et al. (2008) concluded that priming of sunflower achenes with hydrogen peroxide (H_2O_2), salicylic acid (SA), thiourea (TU), gibberellic acid (GA_3) was the best for vigor enhancement compared with other salts and simple hardening.

In semi-arid rainfed areas, particularly in the dominant Mediterranean-type climate, early sowing, rapid seedling establishment and vigorous development could considerably hasten plant growth and may avoid the facing of crucial stages with terminal drought and high temperature stress (Janmohammadi et al., 2013). In recent years, the progress of nanotechnology in combination with biotechnology has appreciably increased the utilization domain of nanomaterials in agriculture fields. Previous studies showed the potential of nanomaterials in improving seed germination and seedling early growth (Zheng et al., 2005; Siddiqui and Al-whaibi, 2014; Sabaghnia and Janmohammadi, 2014). However, the interaction mechanisms at the molecular level between nanoparticles and biological systems are largely unknown. Also, a thorough understanding of the role of nano-sized engineered materials on plant physiology at the molecular level is still lacking. Agricultural utilization of nanoparticles is currently an attractive area of interest. Nanoparticles can easily enter into plant system by overcoming the cell wall barrier in comparison with bulk materials. They have achieved greater consideration because of their highly reactive surface-to-volume ratio property. The recent advances in nanotechnology and its use in the field of agriculture are astonishingly increasing; therefore, it is tempting to understand the role of nanosilicon dioxide ($nSiO_2$) in the germination of seeds.

The purpose of this investigation was to evaluate the effect of seed priming in nanosilica solution on seed germination performance of sunflower to find out the most promising concentration.

Materials and methods

Pre-hydration treatments were including control (non primed seeds) and achenes soaking in solutions containing different concentrations of nano-silica (0.2, 0.4, 0.6, 0.8, 1 and 1.2 mM for 8 h). Achenes of sunflower (*Helianthus annuus* L., cv. Azargol) were used in this experiment. Nanoparticle of silica (SiO_2) was purchased from the Pishgaman Nano, Iran. According to the manufacturer, the particle sizes of SiO_2 ranged from 20 to 30 nm. Specific surface area of nanosized silica was 180-600 m^2 g^{-1} and purity was 99.7%. The result of X-ray analysis of nano silicon dioxide is displayed in figure 1. The high-resolution transmission electron microscopy (HRTEM) image of the nanoparticle sample is shown in figure 2. Mean germination time (MGT) was computed according to Ellis and Roberts (1981) as MGT=Σ*T*i Ni/ΣN*i*, where N*i* is the number of newly germinated seeds at time T*i*. Germination index was calculated as follows: $GI = \Sigma (G_t/T_t)$, where G_t is the number of seeds germinated on day t and T_t is the number of days from beginning of germination test (Hu et al., 2005). The seed lot having greater germination index is considered to be more vigorous.

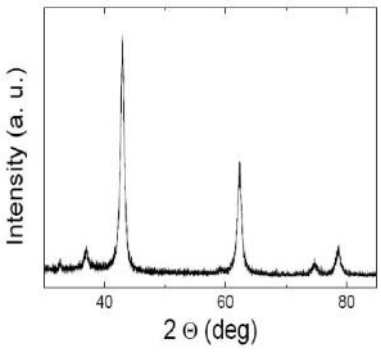

Figure 1. X-Ray diffraction pattern of nano-silicon dioxide particles.

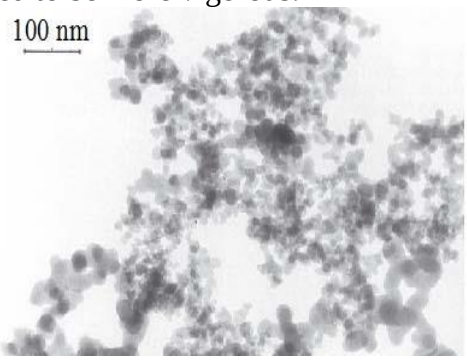

Figure 2. Large area TEM image of silica nanoparticles.

Mean daily germination (MDG), calculated as the cumulative percentage of full seed germination at the end of the test, divided by the number of days from sowing to the end of

the test. The time to 50% germination (T_{50}) was calculated according to the following formula of Farooq et al. (2005). Seedling vigor index based on the length (SVIL) was calculated following modified formula of Ranal and Santana, (2006). Energy of germination (EG) was calculated four day after start of the experiment. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan et al., 2002). Promptness index was calculated according to Ashraf et al. (2006). Data were subjected to analysis of variance (ANOVA) procedures (SAS Institute Inc., 1988), and LSD test was applied at 5% probability level to compare the differences among treatment means.

Results and discussions

Germination index (GI) is suitable indicator for comparisons of germination times. Statistical analysis showed that achene pre-hydration in nanosilica solution could considerably affect the GI. The highest value of this trait was recorded in achenes primed with 0.2 Mm nanosilica solution. However priming in higher concentration of nanosilica could not improve this parameter and even achenes primed at 1.0 Mm concentration showed the lowest value of GI (Table 1). Assessment of mean germination time (MGT) revealed that achene priming with 0.2, 0.4 and 0.8 mM nanosilica solutions could appreciably reduce the MGT as compared to intact achenes. However the highest value of MGT was related to priming at 1.0 Mm concentration. This trend also was recognizable by examining the T_{50} and promptness index (PI). Data with respect to the PI clearly show that PI increased by priming in solution with upto 0.6 Mm concentration of nano SiO_2 and decreased significantly in 0.8 Mm concentration of nano SiO_2 as compared to control. A significant ($P<0.01$) effect of achene pre-hydration treatments was seen on the final germination percentage (GP). Mean compression revealed that pre-hydration in solutions containing 0.2, 0.4 and 0.6 Mm nanosilica cause a slight increase in final germinal percentage, while high concentrations of nanosilica noticeably reduced the germination percentage (Table 1).

Table 1. Effect of seed priming in colloid solution of silica nanoparticles on seed germination characteristics of sunflower (*Helianthus annuus* L.).

Nano-silica Concentration (mM)	GP	MDG	GI	MGT	T_{50}	GR	EG	PI
Control	80.33^{ab}	13.05^{ab}	8.56^{c}	2.52^{bc}	1.71^{b}	0.40^{ab}	73.33^{a}	55.46^{c}
0.2	91.67^{a}	15.27^{a}	12.55^{a}	2.13^{c}	0.38^{c}	0.48^{a}	78.33^{a}	87.00^{a}
0.4	90.00^{a}	15.00^{a}	11.24^{ab}	2.49^{cb}	0.57^{c}	0.41^{ab}	78.33^{a}	82.16^{ab}
0.6	85.00^{a}	14.17^{ab}	8.74^{cb}	2.92^{b}	1.80^{ab}	0.35^{bc}	78.33^{a}	74.16^{b}
0.8	78.33^{ab}	13.05^{ab}	8.32^{c}	2.80^{cb}	1.85^{ab}	0.36^{bc}	58.33^{a}	38.70^{d}
1.00	33.33^{c}	5.55^{c}	2.19^{d}	3.99^{a}	2.18^{a}	0.25^{c}	20.00^{b}	12.80^{e}
1.2	65.00^{b}	10.83^{b}	8.26^{c}	2.23^{bc}	2.09^{ab}	0.45^{ab}	58.33^{a}	37.60^{d}
Level of significance	**	**	**	**	**	*	**	**
CV%	16.49	16.51	17.30	15.71	17.06	15.91	18.32	13.12

GP: germination percentage, MDG: mean daily germination, GI: germination index, MGT: mean germination time, T_{50}: days to 50% germination, GR: germination rate, EG: energy of germination, PI: promptness index. In a column figures with same letter (s) or without letter (s) do not differ significantly whereas figures with dissimilar letter are statistically different. * = Significant at 5% level of probability, ** = Significant at 1% level of probability.

Evolution of mean daily germination (MDG) showed that priming treatments have significant effect on this trait. The response of MDG against pre-hydration treatments was relatively similar to GP so and the best performance was recorded for achenes pre-hydrated in solutions with low concentrations of nanosilica. Generally achenes primed in low concentration of nanosilica solution could finish their germination in a closer spread of times. The contrary trend was observed for germination rate (Table 1). Variance analysis showed a significant effect of priming treatments on energy of germination (EG). Mean comparison revealed that

priming of achenes in high concentration of nano SiO_2 reduced the EG compared with control achenes (Table 1).

Table 2. Impact of pre-sowing seed treatment via the solution of silica nanoparticles on seedling growth of sunflower (*Helianthus annuus* L.).

Nano-silica concentration (mM)	RL	SL	SDW	RDW	SVIL	SVIW
Control	1.08^{c}	7.58^{c}	57.00^{cd}	16.00^{b}	6881^{bcd}	5718^{bc}
0.2	3.15^{ab}	13.37^{a}	86.67^{a}	27.66^{a}	15185^{a}	10562^{a}
0.4	3.36^{a}	10.55^{ab}	82.33^{ab}	30.00^{a}	12527^{ab}	10110^{a}
0.6	2.08b^{c}	8.01^{abc}	63.33^{bc}	16.32^{b}	9021^{abc}	6808^{b}
0.8	1.99^{c}	6.35^{cb}	60.66^{dc}	11.00^{b}	6806^{bcd}	5558^{bc}
1.00	1.58^{c}	3.80^{c}	47.00^{cd}	13.66^{b}	1759^{d}	1947^{d}
1.2	1.43^{c}	4.80^{c}	42.66^{d}	9.33^{b}	4375^{cd}	3320^{cd}
Level of significance	**	*	**	**	**	**
CV%	21.89	30.39	17.49	27.48	24.85	22.93

RL: root length, SL: shoot length, SDW: shoot dry weight (mg), RDW: root dry weight (mg), SVIL: seedling vigor index based on length, SVIW: seedling vigor index based on weight. In a column figures with same letter (s) or without letter (s) do not differ significantly whereas figures with dissimilar letter are statistically different. * = Significant at 5% level of probability, ** = Significant at 1% level of probability.

Results showed that pre-hydration treatment of achenes before germination significantly influenced performance of seedling growth by improving root length, shoot length, shoot dry weight, root dry weight and seedling vigour index (Table 2). Investigation of root length indicated that achene priming in solution with low concentration of nano silica could create the longest roots. However the shortest roots were related to un-primed and primed achenes in solution with high concentration of nano silica (Table 2). Also a significant ($P<0.05$) effect of achene pre-hydration treatments was seen on the shoot length and response trend was similar to status described for root length. Shoot dry weight (SDW) was significantly affected by priming treatments ($P<0.01$). Increasing nano silica concentration resulted in a decrease of SDW (Table 2). However, evaluation of root dry weight (RDW) indicated that significant differences were only recorded for achenes primed in low concentration of nano silica (0.2 and 0.4 mM). Mean comparison of both seedling vigor index suggested that the best performance was related to achenes primed in 0.2 mM solution of nano silica. This status was more evident in SVIL.

Results showed that achenes priming in high concentration of nano silica solution resulted in decreased germination, the present findings seem to be consistent with other research which found that the beneficial influences of the $nSiO_2$ are more pronounced at low concentrations (Sabaghnia and Janmohammadi, 2014; Haghighi et al., 2012). The faster and synchronized germination might be attributed to enhanced metabolic activities in the primed achenes (Wahid et al., 2008). Pre-hydration treatments not only improved the germination rate and time but also enhanced the seedling vigour as indicated by higher values of SVIL, SVIW, root and shoot length and seedling fresh and dry weights.

In semi arid region adverse abiotic stresses, such as high temperatures, soil crusting and shortage of water can reduce the rate or entirely hinder seed germination and seedling emergence in late spring and early summer. In this respect, the application beneficial nano particles for pre-sowing treatments and utilization of seeds with enhanced vigor can be a practicable strategy to obtain healthy seedlings and a better crop stand under unfavorable environmental condition.

Conclusion

Applications of nano-particles can help faster plant germination/production, effective plant protection with reduced environmental impact as opposed to traditional approaches.In conclusion, current results of the reveal that the utilization of nano silicon in germination medium significantly enhanced seed germination potential. However, in order to recognize the possible benefits of applying nano-materials in agriculture, it is important to analyze penetration and transport of nano-particles in the embryo and seedling. Furthermore, the current study invites researchers to evaluate the molecular mechanism involved in invigoration of seed germination after treatments by nano silicon which establishes that nano silicon could be used as a seed coating material or fertilizer for the crop improvement.

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DROUGHT TOLERANCE IN SOME CHICKPEA (Cicer arietinum L.) GENOTYPES UNDER DIFFERENT IRRIGATION REGIMES

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Abstract

Crop drought tolerance issue is one of the most challenging objectives of agronomy and plant breeding investigations. Developing an efficient screening technology and identifying the most important traits contributing toward drought tolerance are major steps in this direction. To go in this quest, an experiment was conducted with seven Kabuli chickpea genotypes were grown under three humidity regimes including (i) rain-fed condition, (ii) full irrigation and (iii) rain-fed condition with two supplement irrigations during flowering and grain filling stages. Analysis of variance indicated significant differences among seven chickpea genotypes for the measured traits. Results revealed that plant height, primary and secondary branches, chlorophyll content, day to maturity, grain yield and yield components were significant. However, there was no significant difference for number of pods per plant, number of seeds per pod, 100-grain weight, grain yield per unit area and grain filling rate. Based on the YP drought tolerance index, genotypes FLIP 03-64C and Azad had the highest yield under non-stressed condition, while genotypes FLIP 98-106C and Azad displayed the highest yield under stressed condition. The relationships among drought tolerance indices are graphically displayed in a plot of two first principal components analysis. Genotypes FLIP 98-106C and Azad are good candidates for commercial recommendation to farmers in both rain-fed and irrigated conditions. Principal component analysis indicated that relative water content, photochemical efficiency of photosystem II, and stomatal conductance were the physiological traits with greater contribution toward drought tolerance and should be evaluated ahead of many other traits in making selections for drought-tolerant chickpea genotypes.

Key words: *drought; genotype; grain yield; rain-fed conditions*

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important cool season pulse crops of dry lands in the world and produced on 700,000 hectares in Iran and ranks fourth in the world after India, Turkey and Pakistan (FAO, 2014). For a wide range of environmental conditions, it is also an alternative pulse crop for increasing the diversification of different cropping systems (Zdemur and Karadavut, 2003). Chickpea yield affected by biotic stresses is quite low, and yield is below its potential. It is traditionally grown as a rain-fed crop on stored soil moisture and this makes terminal drought stress as a primary constraint to productivity. It covers 15% of the cultivated area and contributes to 14% of the world's pulse harvest (FAO, 2014). Besides being an important source of human and animal food, chickpea also plays an important role in the maintenance of soil fertility, particularly in the dry, rain-fed areas.

Irrigated agriculture is the primary user of diverted water globally, reaching a proportion that exceeds highly of the total in the arid and semi-arid areas. The rain-fed agriculture accounts most of the world's farmland and two-thirds of global food production. When water supplies are limited, the farmer's goal should be to maximize net income per unit water used rather than per land unit (Fan et al. 2005). Drought stress is one of the most common adverse environments, which limits crop production in arid and semi-arid areas. It is usually

accompanied by high temperatures, which promote evaportranspiration and hence could accentuate the effects of drought stress (Toker and Cagirgan, 1998). Most crops adapt to drought conditions either through escape, avoidance, or tolerance mechanisms.
Most of chickpea production quantity is done in marginal regions in the spring, and due to lack of rainfall during sensitive periods, terminal drought stress is the major environmental stress for reducing chickpea production in Iran (Sabaghpour et al. 2006). Selection for drought tolerant chickpea genotypes is the most important objective for breeding to drought stress. Breeding for drought tolerance is involved by the lack of fast, screening tools and the inability to create water stress conditions when a large amount of genotypes are to be evaluated. Gain a genetic improvement of seed yield performance under water stressed conditions has been proved to be a difficult task for chickpea breeders while progress in yield performance potential has been much higher in normal condition (Richards et al. 2002).
Stress resistance indices, which provide a measure of drought based on loss of yield under stressed conditions, have been used for screening tolerant genotypes. The genotypes performance under drought stressed and normal conditions seems to be a common starting point in the identification of traits related to drought tolerant and the selection of genotypes for breeding efforts. The stress susceptibility index (SSI; Fischer and Maurer, 1978), the mean productivity (MP; Rosielle and Hamblin, 1981) and tolerance (TOL; Rosielle and Hamblin, 1981), the yield stability index (YSI; Bouslama and Schapaugh, 1984), the superiority index (PI; Lin and Binns 1988), the stress tolerance index (STI; Fernandez, 1992), geometric mean productivity (GMP; Fernandez, 1992), yield index (YI; Gavuzzi et al. 1997), and relative reduction (RR; Sadiki 2006) were proposed to screening drought tolerant genotypes. The objectives of the investigation were to identify drought tolerant genotype(s) of chickpea under rainfed conditions in the semi-arid condition as well as study interrelationships among the resistance indices.

Materials and methods

Seven chickpea genotypes (five newly improved genotypes from International Center for Agricultural Research in the Dry Areas - ICARDA) and two checks cultivars (Arman and Azad from Iran) were used. The experimental fields was ploughed once in early fall and harrowed twice, one week before planting. The experimental design was spilit-plot on the bases of randomized complete block in three replicates. Main plot factor was water regimes (zero irrigation and regular irrigation with irrigation to fill the root zone profile) and sub-plot factor seven chickpea cultivars. Seeds were hand sown on 17 March 2012 in 4 cm depth of soil. In each plot, seeds were sown into 16 rows, at 25-cm row-to-row spacing and 8-cm plant-to-plant spacing. Weeding was done manually. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation. These statistical parameters were calculated:

$$SSI = [1-(Ys/Yp)]/[1-(\bar{Y}s/\bar{Y}p)]$$
$$MP = (Ys+Yp)/2$$
$$TOL = (Yp-Ys)$$
$$YSI = Ys/Yp$$
$$STI = (Yp \times Ys)/Yp^2$$
$$GMP = \sqrt{(Ys+Yp)}$$
$$HM = [2(Yp \times Ys)]/(Yp+Ys)$$
$$YI = Ys/\bar{Y}s$$
$$RR = (Yp-Ys)/Yp$$

where Yp was genotype yield in non-stress conditions; Ys, genotype yield in stress conditions; $\bar{Y}s$, mean yield of all genotypes in stress conditions; $\bar{Y}p$, mean yield of

all genotypes yield in non-stress conditions. The PI (Lin and Binns, 1988) was calculated according to the following formula:

$$Pi = \sum_{j=1}^{n} \frac{(X_{ij} - M_j)^2}{2n}$$

where n is the number of environments, Xij the seed yield of ith genotype in the jth environment and Mj is the yield of the genotype with maximum yield at environment j. Differences were compared by Least Significant Difference Test (LSD) at alpha 0.05. The experimental data were statistically analyzed for variance using the SAS 6.12 (SAS, 1996).

Results and discussion

Based on the SSI, genotypes G2, G3, G5, Arman and Azad were identified as drought tolerance genotypes in stressed condition; while genotypes G1 and G4 displayed the lowest amount of SSI (Table 1). According to MP, genotypes G3 and Azad were found drought tolerance genotypes in stressed condition and genotypes G1, G2, G4 and G5 were identified as drought susceptible ones in stressed condition. Based on the TOL, genotypes G2, G3, G5, Arman and Azad were identified as drought tolerance genotypes in stressed condition; while genotypes G1 and G4 showed the lowest amount of TOL. The genotype G4 was identified as semi-tolerance or semi-sensitive to drought stress conditions (Table 1). According to the stress tolerance index (STI), genotypes G3, Arman and Azad were identified as drought tolerance genotypes; while genotypes G1, G2, G4 and G5 showed the lowest amount of STI (Table 1). Based on the GMP, genotypes G3 and Azad were identified as drought tolerance genotypes in stressed condition while the other remained genotypes displayed the lowest amount of GMP (Table 1). Mevlut and Sait (2011) showed that genotypes with high STI values usually have high difference in yield in two different humidity conditions. They reported relatively similar ranks for the genotypes were observed by GMP and MP parameters as well as STI, which suggests that these three parameters are equal for screening drought tolerant genotypes.

According to the harmonic mean (HM), genotypes G3 and Azad were identified as drought tolerance genotypes while the other remained genotypes showed the lowest amount of GMP (Table 1). The results of both GMP and HM indices were completely similar. It seems that this similarity is due to nature of their calculating formulas and so it is logical to use one of them in the future studies. Based on the YI index, genotypes G3 and Azad were identified as drought susceptible genotypes; while genotypes G2, G4, G5 and Arman showed the lowest amount of YI and were drought tolerance genotypes (Table 1). According to the PI index, genotypes G1, G4 and G5 were identified as drought susceptible genotypes; while genotypes G2, G3 and Arman showed the lowest amount of PI and were drought tolerance genotypes (Table 1). According to the YSI index, genotype G1 was identified as drought tolerance genotypes; while genotypes G2, G5 and Arman showed the lowest amount of YSI as drought tolerance genotypes (Table 1). Based on the RR index, genotypes G2, G5 and Arman were identified as drought tolerance genotypes in stressed condition while the genotype G1 displayed the lowest amount of RR (Table 1). According to the K_1STI index, genotypes G2, G3, Azad and Arman were identified as drought tolerance genotypes in stressed condition while the genotypes G1, G4 and G3 showed the lowest amount of K_1STI (Table 1).

Table 1. Mean yield performances of chickpea genotypes in non-stressed (YP) and stressed (YS) conditions and twelve drought resistance indices.

Cultivar	Code	YP	YS	SSI	MP	TOL	STI	GMP	HM	YI	PI	YSI	RR	K_1STI	K_2STI
FLIP 03-71C	G1	1436.8	873.2	0.572	1155	563.5	0.00071	1119.9	1085.9	1.057	220371	0.607	0.393	0.00044	0.00079
FLIP 03-64C	G2	1922.2	709.8	1.23	1316	1212.4	0.0008	1167.7	1036.3	0.859	91997	0.371	0.629	0.00094	0.00061
FLIP 98-106C	G3	2095.3	1015.2	1.096	1555.2	1080.2	0.00095	1454.5	1361.3	1.229	27259	0.491	0.509	0.00129	0.00143
FLIP 00-40C	G4	1515.3	759.9	0.767	1137.6	755.3	0.00069	1072.5	1011.2	0.92	206476	0.5	0.5	0.0005	0.00062
FLIP 99-66C	G5	1679.4	739.8	0.954	1209.6	939.6	0.00074	1114.6	1027	0.895	141751	0.441	0.559	0.00064	0.0006
Arman	G6	1997.1	744.6	1.271	1370.9	1252.5	0.00084	1219.3	1084.6	0.901	69929	0.373	0.627	0.00105	0.00071
Azad	G7	2033.4	939.5	1.11	1486.4	1093.9	0.0009	1380	1281.7	1.137	38403	0.466	0.534	0.00116	0.00117

To better understand the relationships among the Stress tolerance indices, a principal component analysis (PCA) based on the rank correlation matrix was performed. The fitted PCA model indicated that, the first two PCAs explained 95% (59 and 35% for PCA1 and PCA2, respectively) of variation for stress tolerance indices. Considering high magnitude of explanation, the decomposition into PCAs would be useful to understand and explore the relationship among genotypes and indices. The relationships among the different statistics are graphically displayed in a biplot of PCA1 and PCA2 (Fig. 1). In this biplot, the PC1 axis mainly distinguishes the methods of RR and YSI from the other methods (we refer to these as Class 1 (C1) methods) and genotypes G3, G3 and G7 from the other genotypes. The second PCA axis separates YP, K1STI, TOL and SSI (we refer to these as Class 2 (C2) methods) from the PI, STI, MP, GMP, HM, K2STI, YI and YS (we refer to these as Class 3 (C3) methods). Mean yield under normal condition (YP) also groups near Class 2 methods, while mean yield under stressed condition (YS) groups near Class 3 methods. Also, genotypes G2 and G5 group near Class 2 methods and genotypes G1 and G4 group near Class 3 methods.

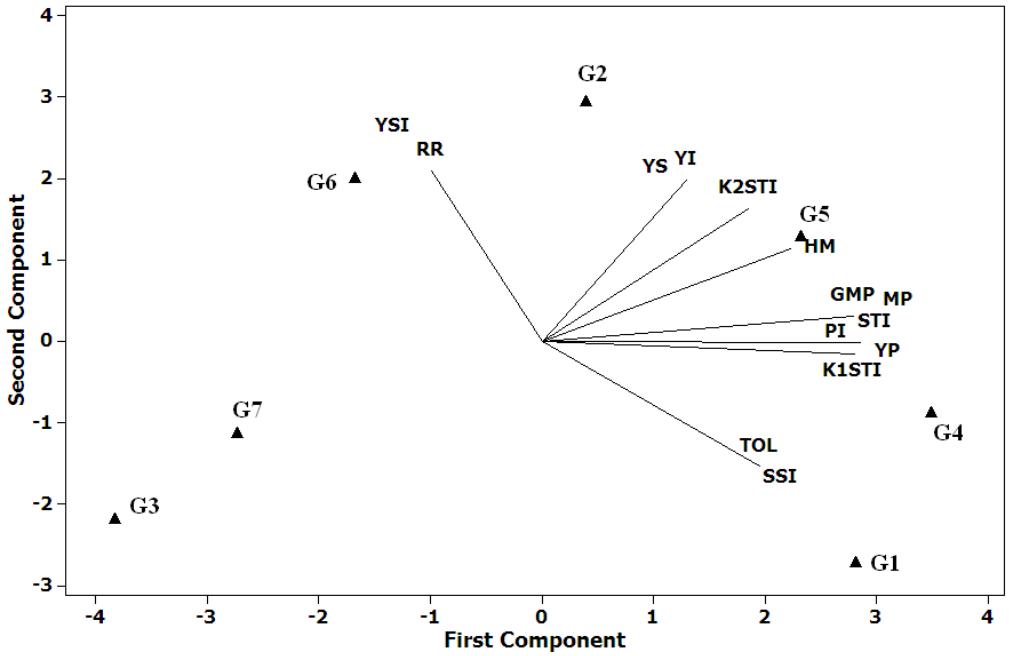

Fig. 1. BIPLOT based on PCA1 and PCA2 scores of drought tolerance indices and genotypes in chickpea.

Ehdaie and Shakiba (1996) found no correlation between stress susceptibility index and yield under optimum condition. The observed relationships in our chickpea genotypes were consistent with those reported by Golabadi et al. (2006) in durum wheat, Khalili et al, (2012) in canola. Several studies indicated that GMP, STI, PI and MP indices are preferred in late drought condition for selecting the most favorable genotypes (Sio-Se Mardeh et al. 2006; Akcura et al. 2011). The HM and K_2STI could be used for screening drought tolerant high yielding genotypes in the both conditions. Such similar conclusion is reported by Mohammadi et al. (2010). The mentioned parameters under level of high to moderate stress were correlated Akcura et al. (2011) with yield under both conditions. Also, they were able to differentiate genotypes belong to genotypes with high yield performance in both conditions, from the others.

Based on biplot of PCA, the indices of STI, K_1STI, MP, GMP, and PI exhibited strong correlation with YP, while YI showed strong correlation with YS, therefore, YS can discriminate drought tolerant genotypes with high grain yield under stress conditions. Also, K_2STI and HM exhibited relatively moderate association with both YP and YS, thus, they can discriminate drought tolerant genotypes with high grain yield at the same manner under stress and non-stress conditions.

Conclusion

Chickpea-sowing strategies vary with environment so as to fit the crop into the farming system and minimize exposure to the prevalent stresses. In semi arid region of Iran with Mediterranean climate Where is characterized by the lowest, most winter- dominant annual rainfall, and the highest summer temperatures management of the irrigation and selection of drought tolerant genotypes is very important. Although in these areas supplemental irrigation can ameliorate drought stress, screening drought tolerant cultivars using ranking method, three dimensional plots should not be ignored. Among different resistance and tolerance indices were evaluated STI, K_1STI, MP, GMP, and PI have high correlation with grain yield under stress and non-stress condition indicating more suitability of these indices for selection of resistant genotype. It is further concluded that genotype G3 and cultivar Azad have uniform superiority under both stress and irrigated conditions. The genotype G3 (1015 kg ha^{-1} at stressed conditions) besides cultivar Azad (940 kg ha^{-1} at stressed conditions) were found to be the most favorable genotypes and are thus recommended for farmers in arid and semi-arid areas of Iran.

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PRODUCTION OF FLAVONOIDS IN HAIRY ROOT CULTURES OF TEUCRIUM POLIUM USING FUSARIUM GRAMINEARUM EXTRACT AS ELICITOR

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Abstract

Teucrium polium L. is an important medicinal plant, which has been used for over thousands of years in traditional medicine. It possesses various medicinal properties such as antidiabetic and anticancer effects. The plant is rich in secondary metabolites, such as flavonoids and sesquiterpenes. Flavonoids have a wide distribution in plants and diverse biological activities including antioxidant, antimicrobial, anti-inflammatory effects. Secondary metabolites in plant cells are present in very small quantities. Hairy roots production a valuable system for studying secondary metabolites biosynthesis and production is large-scale drug compounds. Different biotechnological strategies such as elicitation are currently being for increasing metabolites biosynthesis in hairy root cultures. In this research, the effect of various concentrations (0, 0.5, 1, 1.5% v/v) of *Fusarium graminearum* extract (FE) on production flavonoids (FVs) was assayed. The elicitor was added to the hairy root cultures on day 28 and hairy roots were harvested 12 days after an exposure time of 48 h. FE at a concentration of 1% induced the highest increase of FVs (7.41 mg/g DW), which were 12.14-fold, higher than the level in non-elicited control. The enhancing effects of FE on FVs was correlated with increased activity of the enzyme phenylalanine ammonia-lyase (PAL), and the expression of *PAL* gene involved in the biosynthesis of this compounds. These results suggested potential on-demand production of in vitro FVs using elicitation in *T. polium* hairy root cultures.

Keywords: *Teucrium polium, elicitation, Fusarium graminearum, hairy root culture, flavonoid*

Introduction

Human being has used medicinal plants since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led the discovery of novel drug candidates used against diverse diseases. *Teucrium polium* L. (known popularly as felty germander), belongs to the Lamiaceae family, is one of the wild-growing flowering herb native to Iran and the Middle East as part of the natural flora (Abdollahi et al., 2003). As a traditional remedy, *T. polium* is used for treatment of convulsion, headache, and digestive diseases in Iran (Baluchnejadmojarad et al., 2005). There are also some reports in the literature for antioxidant effects of crude extract of *T. polium* (Ljubuncic et al., 2006). Its antioxidant activity refers to the presence of flavonoids, iridioids, crisiols, volatile oils (Afifi et al., 2005).

Hairy roots produced by *Agrobacterium rhizogenes* infection are characterized by fast growth, biosynthetic stability, and ability to grow in hormone–free medium, all advantages that make transformed roots efficient sources for secondary metabolite production (Srivastava et al., 2007).

Secondary metabolite biosynthesis in plants depends on environmental stresses; precursors and elicitors can stimulate their accumulation. Elicitors are biological (components of microbial cells and poly- and oligosaccharides), chemical (heavy metals, pesticides, and the signaling compounds in plant defense responses), or physical (cold shock, UV, hyperosmotic stress, ultrasound, and pulsed electric field) factors that induce enzymatic activity against stress (Harsh et al. 2003). Use of fungal elicitors is an important way to enhance secondary

metabolites production in plant tissue cultures. These elicitors were utilized mainly in the form of living or autoclaved mycelia, crude extracts, peptides, proteins, and polysaccharides. Fungal elicitors have successfully stimulated production of many valuable bioactive compounds (Ming et al. 2013).
To the authors' knowledge, there were no previous reports about the effects of fungal elicitors on the growth and flavonoid production in *T. polium* hairy root cultures. Therefore, in this study we investigated the effect *of Fusarium oxyxperium* extract on flavonoid production in hairy root cultures of *T. polium*.

Materials and methods

Hairy root culture

The *T. polium* hairy roots used in this work were derived after infecting the leaf explants with a mikimopine type strain of *Agrobacterium rhizogenes* (A13). Stock cultures of the hairy roots were maintained on solid, hormone-free half-strength B5 medium with 7.5 g/l agar and 20 g/l sucrose, at 25 °C in the dark. All experiments were carried out in shake-flask cultures with 250 ml Erlenmeyer flasks on an orbital shaker set at 25 °C and 180 rpm.

Extract of mycelium preparation and induction

Fusarium graminearum extract (FE) was prepared as described by Esmaeilzadeh et al. (2011). For the elicitation treatment, 28-day-old hairy root cultures of the TP-HR-2 line were inoculated into 100-ml Erlenmeyer flasks containing 25 ml of liquid MS medium supplemented with various concentrations of FE (0, 0.5, 1, 1.5% v/v) at the exposure time of 48 h. The hairy root culture without FE treatment was used as a control. All cultures were incubated on an orbital shaker at 100 rpm in darkness at 25°C. At the end of the exposure time, the root cultures were cultured in a fresh medium and subjected to total flavonoid content analysis after 12 days of elicitation. Growth rate of elicitor-treated hairy root cultures was also determined in term of dry weight (DW, mg/30 ml culture medium). For DW measurement, the samples were lyophilized.

Determination of total flavonoid content

About 100 mg of dry hairy root sample powder was macerated with 2 ml of methanol and kept overnight at room temperature. The extract was collected after filtration using Whatman No.1 filter paper and was dried under reduced pressure at 40 °C using a rotary evaporator. Total flavonoid content was determined using the aluminum chloride colorimetric method (Chang et. al., 2002). Rutin was used for establishing the calibration curve. The total phenolic content was expressed as mg rutin equivalents per 100 g of DW.

Measurement *PAL* gene transcripts and phenylalanine ammonia lyase activity

Total RNA was isolated from the elicited hairy roots using RNeasy plant mini kit (Qiagen, Germany) according to manufacturer's instructions. RT-PCR was conducted using Titan One-Tube RT-PCR Kit (Roche applied science, Germany) according to the manufacturer's instructions. The primer pair used to amplify of the *PAL* gene was: 5′-GACGCTGCTGGGGCCTTCA-3′ (forward) and 5′-GGCGTCAAAAAGCACCATGGAG-3′ (reverse). For a semi-quantitative RT-PCR to quantify the relative transcript levels of target gene, the 18S rRNA transcript was amplified using primer pair 5′-TGTT

GGCCTTCGGGATCGGAGTA-3′ (forward) and 5′-GCTTTCGCAGTTGTTCGTCTTTCA-3′ (reverse). RT-PCR analysis was performed at least three replicates and relative quantification of expression level was calculated as the *PAL*/18S rRNA ratios using with Image J software (NIH).

The phenylalanine ammonia lyase (PAL) was extracted from the fresh hairy root of *F. tataricum* with borate buffer (pH 8.8). The PAL activity was determined based on the conversion of L-phenylalanine to cinnamic acid according to the published method of Wu et al. (2002).

Statistical analysis

All experiments were carried out in triplicate. The data were submitted to analysis of variance (one-way ANOVA) to detect significant differences by the Statistical Analysis System (SAS 9.1) programme package. The means were compared using Duncan's multiple rage test (DMRT) at the 5% probability level.

Results and discussion

Effect of FE on growth and flavonoid production

The time course of FE-treated hairy root growth was evaluated to determine the exact time of elicitor treatment. The kinetics of hairy root growth exhibited a slow growth period in the first week, a rapid, linear growth period between the third-forth weeks, achieving the highest biomass concentration of 1200 mg/ml on 28^{th} days and a stationary phase began after the fourth week with a declining of biomass rate. It has been demonstrated that elicitor treatment at late log phase results in higher biomass yield as well as secondary metabolite production (Chandra and Chandra, 2011). Therefore, we determine the 28^{th} days of growth kinetic as the suitable time of elicitation. All the concentrations of FE were shown to increase the growth of airy roots in a dose-dependent manner. The biomass of *T. polium* hairy roots was intensified compared to non-elicited control (Fig. 1). FE a 1.5% increased the growth of hairy roots by 44%. There are a few reports about the elicitation effect of *F. graminearum* on biomass production and secondary metabolites accumulation in medicinal plants. The enhancement of biomass production *by F. graminearum* was also seen in *Valeriana officinalis* hairy root cultures (Dini Torkamani et al., 2014). In the opinion, the putative mechanism for the plant growth promotion by fungi extracts might be that fungi elicitors induce the local and systemic defence responses of hairy roots (Ming et al., 2013).

The effect of *F. graminearum* extract (0.5–1.5% v/v) on flavonoid production is shown in Fig. 2. FE at concentration of 1% gave a higher content of total isoflavonoid (7.41 mg/g DW) after 12 days of treatment. This level of flavonoid content were 12.14-fold higher than that of non-elicited control (0.61 mg/g DW). Esmaeilzadeh et al. (2011) reported that *F. graminearum* cell extract induced the highest increase of PTOX in *L. album* cell culture in compared with the four other fungal extracts. In another study, the culture filtrate of *F. graminearum* exhibited promoting effects on flavonols and flavonoids content of the *L. album* cell culture (Tahsili et al. 2014).

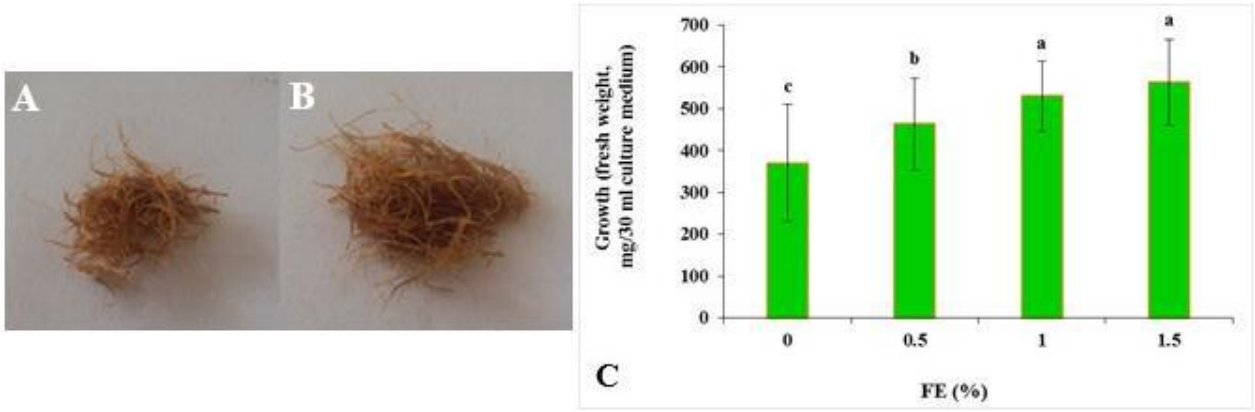

Fig. 1. Effect of *F. garminearum* extract (FE) on biomass production in hairy root cultures of T. *polium*. A: non-elicited root, B: hairy root after treatment with FE (1%), C: growth rate of hairy roots upon treatment with FE.

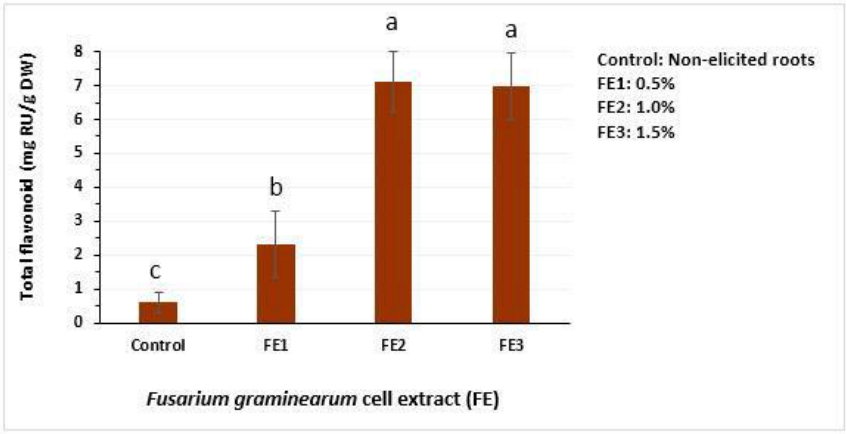

Fig. 2. Effect of *F. graminearum* cell extract on total flavonoid content of hairy roots of *T. polium*. All data are presented as the means ± SD with n = 3. Means with different letters are significantly different (P < 0.05) according to DMRT.

The exposure time of an elicitor is important for effective elicitation of secondary metabolites (Vasconsuelo and Boland (2007). We used a low exposure time (48 h) for elicitation of flavonoids in *T. polium* hairy root culture using *F. graminearum* extract. Similarly, Tahsili et al. (2014) and Kumar et al. (2012) reported an exposure time of 48 h as optimum time of treatment for induction of secondary metabolites production in *L. album* with *F. graminearum* and *P. indica* cell filtrates, respectively. Elicitor concentration and the time of incubation with elicitor as well as the culture conditions and growth stage of the cultured cells are decisive for the elicitation process (Sivanandhan et al. 2013).

Effect of *F. graminearum* cell extract on *PAL* gene expression and enzyme activity We found a high expression of the *PAL* gene in this culture period (Fig. 3). Gene expression in the treated root cultures was increased after the addition of FE, which was 4.26-fold higher than that of the control at 1% of FE (Fig. 3).

PAL, which acts as the bridge between primary metabolism and natural product biosynthesis, catalyzes the first step of phenylpropanoid pathway, i.e. deamination of phenylalanine to cinnamic acid (Kumar et al. 2012). In the present study, total flavonoids were increased in response to the fungi cell extract. With increasing in these compounds, also PAL activity was enhanced after elicitation (Fig. 4). A maximum increase of 3.22-fold in PAL activity (12.26 µM cinnamic acid/mg protein/min) corresponding to 12.14-fold increase in flavonoid content (7.41 mg/g DW) was obtained when FE at a level of 1% was added to hairy root cultures. A correlation between changes in the levels of phenylpropanoid biosynthetic enzymes and product accumulation (Nicholas et al. 1994).

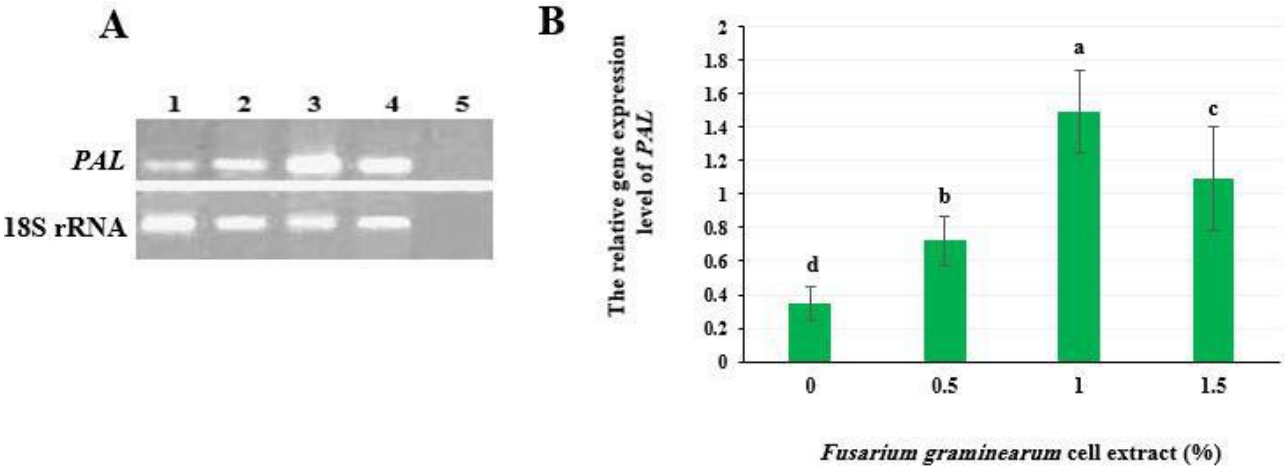

Fig. 3. Dose-dependent transcript level of PAL gene in hairy root cultures of *T. polium* 12 days after treatment with *Fusarium graminearum* cell extract. Relative quantification (B) indicates the *PAL*/T18S rRNA ratio calculated with Image J software (means ±SD, n = 3). 1: non-elicited hairy root, 2-4: hairy roots elicited with 0.5-1.5% of the fungi cell extract, respectively. 5: non-template RT-PCR reaction as negative control

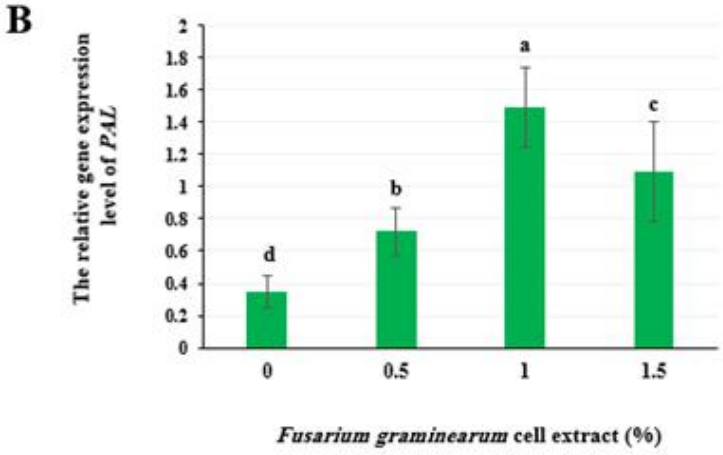

Fig. 4. The phenylalanine ammonia lyase (PAL) activity of *T. polium* hairy root cultures after elicitation treatment with different concentrations of *Fusarium graminearum* cell extract. Fungi cell extract was applied to the hairy root cultures on day 28, and the treatment period was 48 h; Values are presented as means ±SD, *n*=3. Different letters indicate significant differences a t $P < 0.05$ according to the Duncan test.

Conclusion

To the best of our knowledge, there were no previous reports on the use of fungi elicitors for promoting the hairy root growth and flavonoid production of *T. polium*. Without obvious

changes in the appearance of the hairy roots, the exogenous fungal mycelia crude extracts effectively stimulated the flavonoid biosynthesis of *T. polium* hairy root, and the stimulation effect was closely with elicitation concentration. Furthermore, the gene expressing levels were consistent with total flavonoid accumulation levels. These promising results provide a hope for industrial level production of flavonoids or other pharmaceutical compounds from *T. poilum* using elicitation.

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THE EFFECT OF FOLIAR FERTILIZATION ON THE CHEMICAL CONTENT OF KERNELS OF FERANJEZ ALMOND CULTIVAR GROWN IN VALANDOVO

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Abstract

The effect of foliar fertilization on the chemical content of kernels from *feranjez* almond cultivar in Valandovo region in the period of the year from 2012 to 2013 was determined. The experiment was set in four variants and three repetitions. The variants were: Control (untreated); Ever green co Me (55% organic matter, 2% w/w Mg, 2% w/w Fe, 2% w/w Zn, 2% w/w Mn, 0.5 % w/w Cu, 0.5% w/w B); Biolinfa (34% organic matter 3% N, 5.80% K_2O) and Oligomix (1.20% B, 0.10% Cu, 4% Fe, 1.50% Mn, 0.10% Mo, 2% Zn).
The distance of fruit planting was 4.5 m row by row and 3.5 m in the rows. In each variant and repetition were included 60 plants, and total in all experiment were involved 720. Three foliar treatments were applied with given above fertilizers at a concentration of 0.4%. In the end of the November, soil fertilization with the fertilizer Polyfeed 11-44-11+ME (Fe, B, Zn, Mn, Co, Mo) in quantity amount of 450 kg/ha were done.
Before setting up the experiment, soil agrochemical analyses were made, and was concluded good fertility with nitrogen, medium fertility with phosphorus and potassium. The foliar fertilization has a positive influence on the chemical content of almond kernels. The highest average nitrogen content (3.66%), phosphorus (1.11%), potassium (1.15%) and oils (57.40%) were determined in the kernels from variant Ever green co Me (55% organic matter, 2% w/w Mg, 2% w/w Fe, 2% w/w Zn, 2% w/w Mn, 0.5 % w/w Cu, 0.5% w/w B).
The highest average content of calcium (0.92%) and magnesium (0.39%) were determined in the kernels from variant Oligomix (1.20% B, 0.10% Cu, 4% Fe, 1.50% Mn, 0.10% Mo, 2% Zn).
Key words: *almond kernels, foliar fertilization, oils.*

Introduction

The aim in the modern agriculture is to get higher yield that will characterize with better quality. Plant nutrition is one of the most important agrotechnical measures, that together with the others have to allow uninterruptible, high and economically production (Datnoff et al., 2007). The right plant nutrition regime is necessary for normal growth, yield and getting quality product (Domagalski et al., 2008). It means available of all macro and micro biogenic elements in appropriate phenophase of plant growth. Each biogenic element has its specific influence on different parts of the plants. Plant nutrition has an influence on numerous physiological – biochemical processes, that affecting growth, development and yield (Dzamič and Stevanović, 2000; Glintic and Krstic, 1990). Plants that have timely and right nutrition are getting fruits with characteristic shape, color, size and with typical organoleptic properties. Limited of the nutritious elements is happened because of the different reasons. Intensive agriculture and use of high productivity cultivars led to a continuous decrease in soil micronutrient content (Jekić snd Brković, 1986; Sarić et al., 1989).
Using of foliar fertilizers the fruit cultures nutrition, has a big importance in getting higher yields as well as products with better quality. Foliar fertilizers allow direct supplying of leafs,

flowers and fruits with nutritious elements in a period when they are necessary. Foliar spray with fertilizers is necessary to further activity in the whole system of optimal mineral nutrition of plants (Kostadinov and Kostadinova, 2014). It provides more economical water regime of plants and allows overcoming the physiological disturbances caused by adverse soil conditions that hamper mobility and nutrients absorption.

In unregularly soil conditions, unregularly pH value, low or high soil temperature, fixation in different nutrients, the root cannot adopt the nutrients at all (Sarić et al., 1986; Šaćiragić and Jekić, 1988). In such cases, the foliar nutrition has an important influence. It is an additional nutrition and measure that allow rapid and efficiency effect of correction of the plant nutrition (Velićković, 2002).

The almond, *Amygdalus communis*, is kernel fruit that bellows at the family of *Rosaceae*, and under family *Prunoidae*. It is old fruit culture that is counts in economically importance kernel fruit and has numerous positively characteristic, too.

The importance of almond is because of the kernel, which is very reach in fats, proteins, mineral matters, sugars, cellulose, vitamins and amino acids (Bulatović, 1985). On the other hand, the almond has a big value for human nutrition, pharmaceutics, cosmetic industry and so.

The aim of our explorations was to obtain the influence of soil and foliar fertilization on the chemical content of kernels by almond cultivar *feranjez*, grown in Valandovo region.

Materials and methods

The field experiment with almonds was set in Valandovo region (Republic of Macedonia), during the years of 2011 and 2012. During the field experiment setting, the fruits were 7 years old. The material of work was almond cultivar *feranjez*.

The planting distance was 4.5 m row by row and 3.5 m in the rows. The nutritional area were 15.75 m^2, i.e. 635 fruits/ha.

In the exploration were included 4 variants in 3 repetitions. In each variant and repetition were included 60 plants, or total in whole experiment the number of plants was 720.

The field experiment was set in terms of watering in system drip. During the almond vegetation period were applied all basic agricultural measures.

Variants in experiment were:

Control (untreated);

Ever green with Me (55% organic matter, 2%w/w Mg, 2%w/w Fe, 2%w/w Zn, 2% w/w Mn, 0.5 %w/w Cu, 0.5 %w/w B);

Biolinfa (34% organic matter, 3 %N, 5.80 % K_2O);

Oligomix (1.20 % B, 0.10 %Cu, 4 % Fe, 1.50 % Mn, 0.10 % Mo, 2 % Zn).

The soil fertilizing was applied in the end of November and the fertilizer Polyfeed 11-44-11+ME (Fe, B, Zn, Mn, Co, Mo) was used in the quantity amount of 450 kg/ha.

Each variant and repetition were treated foliar with 0.4% solution of the tasted fertilizers. The application of fertilizers was done with manually spraying the played leaves. The treatments were made in the evening hours. During the vegetation period were conducted 4 foliar treatments. The first treatment was made 10-15 days before flowering, and the other treatments was made after flowering at a distance of 15-20 days.

The harvesting was carried out separately by variants and repetitions.

Before setting up the experiment soil samples were taken for agrochemical analyses and were performed on the following parameters:

pH value - determined potentiometric with pH meter (Bogdanović et al., 1966);

Content of easy available nitrogen – determined by method of Tjurin and Kononova;

Content of easy available phosphorus – determined by AL method and reading of spectrophotometer (Bogdanović et al., 1966);

Content of easy available potassium – determined by AL method and reading of spectrophotometer (Bogdanović et al., 1966);
Content of humus – determined by permanganese method of Kotzman (Bogdanović et al., 1996)
Content of carbonates–determined with Schaiblerov Calcimeter (Bogdanović et al., 1966).
In the kernels were determined the following parameters:
The content of nitrogen (N) - determined using Kjeldal method (Sarić et al., 1989);
The content of phosphorus (P_2O_5) - determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1989);
The content of potassium (K_2O) - determined by incineration of the material with concentrated H_2SO_4 and phlamenphotometar (Sarić et al., 1989);
The content of calcium (SAT) - determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1989);
The content of magnesium (Mg) - determined by applying atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1989);
The content of oil was determined with Sokslet method (Sarić et al., 1989);

Results and discussion

Climate is one of the most important environmental factors from which successful raising depends of all fruit kinds with no exception of almonds (Šoškić, 1996).
The influence of climate elements manifest as time of vegetation of fruit as well as in the phase of resting.Almonds best gain a head in regions with short winter without big temperature variants with absents of frost in annual amount of average temperature over 3000^{o}C (Bulatović, 1989). Valandovo region is known as region with lots of shiny days. Temperature requirements of almond for growth and development in the period of standby are large. It is enough in sequel of 100 hours, the temperature to move from 0 to 6^{o}C for almond awakening. Average year temperature of the air in the Valandovo region is 15^{o}C. Sensibility of low temperatures is brand characteristic. Almond varieties that blossom early are more sensitive than other. Unopened blossoms can be damaged on -3^{o}C to -4^{o}C, and open on -1.5 to - 2.8^{o}C, and just planted fruit on - 1 to -1.5^{o}C (Bulatović, 1985). Almonds are sensitive on very high atmospheric humidity and suffer from diseases and don’t give good yields. Average year relative humidity in Valandovo region is 71 % with maximum of 80 % in November, December and January. Soil conditions have an especially importance on growing, developing and fruits quality. The almond has the best growth and yield in deep, alluvial - diluvial, loamy - sandy, humus carbonate soils with significant content of lime (Ubavić et al., 2001). Particularly suitable are soils with neutral reaction and good penetration of water and air. Salty and acidic soils, too wet and clay are unsuitable for growing almonds.

Table 1. Agrochemical analyses of soil

Lab. No.	Depth cm	pH		Available mg/100g soil			Humus %	$CaCO_3$ %
		KCl	H_2O	N	P_2O_5	K_2O		
1	0-20	7.14	7.62	14.70	6.60	20.00	1.86	/
2	20-40	7.19	7.98	15.26	6.20	17.00	1.90	/
3	40-60	7.05	7.86	11.48	5.00	17.00	1.80	2.50
	Average 0-60	**7.13**	**7.83**	**16.20**	**5.93**	**18.00**	**1.85**	**0.83**

Table 2. Chemical content of kernels in % of dry matter (average 2011/2012)

Variant	N	P_2O_5	K_2O	CaO	MgO	Oils
1	3.25	0.99	0.99	0.84	0.34	55.15
2	3.66	1.11	1.15	0.88	0.37	57.40
3	3.53	1.04	1.08	0.89	0.37	55.50
4	3.39	1.02	1.06	0.92	0.39	53.70

LSD (0.05) = 0.13 LSD (0.05) = 0.04 LSD (0.05) = 0.06 LSD (0.05) = 0.03 LSD (0.05) = 0.02
LSD (0.01) = 0.10 LSD (0.01) = 0.06 LSD (0.01) = 0.08 LSD (0.01) = 0.04 LSD (0.01) = 0.03

From data shown in Table 1, can be concluded that soil in which the field experiment was set, has neutral pH value, good fertility with available nitrogen, but a medium fertility with available phosphorus and potassium. It has medium fertility with humus, too. There is low presence of carbonates.

From data shown in Table 2, can be concluded that soil and foliar fertilization have positive influence on the chemical content of kernels. In all of the variants treated with different kinds of fertilizers, analyzed parameters have higher value compared with the control, untreated variant.

The highest average nitrogen content (3.66%), phosphorus (1.11%), potassium (1.15%) and oils (57.40%) is determined in kernels in variant 2.

For the nitrogen content, statistically significance differences are determined in all of the variants at both levels. For the phosphorus content statistically significance differences at LSD 0.05 level is determined in variants 2 and 3, and at LSD 0.01 level in the variant 2. For the potassium content statistically significance differences at LSD 0.05 level is determined at all variants, and at LSD 0.01 level in the variant2 and 3.

The highest average content of calcium (0.92%) and content of magnesium (0.39%) is determined in the kernels from variant 4. For calcium and magnesium content are obtained statistically significance differences at LSD 0.05 level in variants 2, 3 and 4. At LSD 0.01 level for the content of calcium are obtained significance differences in variants 3 and 4, and for the magnesium content in the variant 4.

Higher content of tested elements in all of the variants, compared with the control variants is a result of the chemical content of used foliar fertilizers as well as their absorption in the plant organs, i.e. kernels.

Macro and micro biogenic elements in the content of used fertilizers have an influence on numerous physiological – biochemical processes that are of vital importance in plant vegetation cycles.

Conclusion

Based on the obtained results for the influence of foliar fertilization on the chemical content of almond kernels from *feranjez* cultivar grown in Valandovo region, the following conclusion can be made:

The soil where the field experiment were carried out, was characterized with neutral pH value, good fertility with available nitrogen and medium fertility with available phosphorus and potassium;

In all of the variants treated with foliar fertilizers, in kernels were obtained higher content of all tested elements compared with the control variant;

The highest average content of nitrogen (3.66%), phosphorus (1.11%), potassium (1.15%) and oils (57.40%) were determined in kernels from the variant 2 (Ever green with Me (55% organic matter, 2%w/w Mg, 2%w/w Fe, 2%w/w Zn, 2% w/w Mn, 0.5 %w/w Cu, 0.5 %w/w B));

The highest average content of calcium (0.92%) and magnesium (0.39%) was determined in kernels from variant 4 (Oligomix (1.20 % B, 0.10 %Cu, 4 % Fe, 1.50 % Mn, 0.10 % Mo, 2 % Zn);
For the nitrogen content, statistically significance differences are determined in all of the variants at both levels. For the phosphorus content statistically significance differences at LSD 0.05 level is determined in variants 2 and 3, and at LSD 0.01 level in the variant 2;
For calcium and magnesium content are obtained statistically significance differences at LSD 0.05 level in variants 2, 3 and 4.

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THE EFFECT OF PLANT GROWTH REGULATORS ON MORPHOGENESIS IN TISSUE CULTURE OF SOME AGRICULTURE SPECIES

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Abstract

The vegetative propagation of the plants in *in vitro* conditions enables to abbreviate the process of selection, enhance the genetic stability of plants and improve the production of healthy plants free of viruses.

In this paper the results of our experimental work for determination of the potential of *in vitro* morphogenesis and micropropagation of some agricultural species are presented (*Apium graviolens* L., *Daucus carota* subsp. *sativus* L., *Cucumis sativus* L., *Cucurbita pepo* var. *cylindrica, Raphanus sativus* var. *radicola,* and important agricultural species from *Apiaceae, Brassicaceae, Cucurbitaceae* and *Solanaceae* family). Different initial explants were cultivated *in vitro* on various media supplied by different combinations and concentration of plant growth regulators. The main objective of this research was to set up meristem tissue culture and non-meristem explants, to explore the properties of the tissues *in vitro*, and to observe their possibilities for morphogenesis and micropropagation.

Keywords: *in vitro, vegetative propagation, phytohormones, regeneration, micropropagation.*

Introduction

Biotechnological approaches like micropropagation, somaclonal variation, *in vitro* conservation, protoplast fusion, and development of novel transgenic plants have great potential in conservation, utilization and increasing the production of spices. Efficient micropropagation systems are available for many spices which are being used for propagation, conservation, safe movement and exchange of germplasm, crop improvement through somaclonal variation and transgenic pathways (Babu et al., 2015).

At the peak of the plant tissue culture era in the 1980s, in a relatively short time, many commercial laboratories were established around the world to capitalize on the potential of micropropagation for mass production of clonal plants for the horticulture industry. Today plant tissue culture applications encompass much more than clonal propagation (Akin-Idowu et al., 2009).

Micropropagation has been employed a large scale production of decease free planting material and germplasm conversation. High rate of multiplication coupled with the additional advantage of obtaining diseases free planting material makes micropropagation a viable alternative to a conventional propagation.

Morphogenesis in plants is a complex phenomenon and is being regulated by numerous factors and in-between relationships of plant organs, tissues and cells also. The correlation between cells tissues and organs of a plant plays important role in the growth and development in *in vivo* and in *in vitro* conditions. The study of such complex system can be simplified with cell, tissue or organ isolation and their cultivation *in vitro*. In such conditions, the influence of certain factors on the organogenesis and differentiation of the plant tissue can be traced (Koleva Gudeva and Trajkova, 2012). Even today much more is not fully understood about micropropagation, and morphogenesis in tissue culture, and although general guidelines for micropropagation have been established, each plant species is unique.

Despite this problems, there are e large number of species being micropropagated on a commercial scale throw the world (Kitto, 1997).
This paper surveys the effect of plant growth regulators as an important role in the plant morphogenesis of some important agricultural species form *Apiaceae, Brassicaceae, Cucurbitaceae* and *Solanaceae,* as a main factor for successful commercial micropropagation.

Material and methods

This study was conducted in the Laboratory for Plant Biotechnology at Faculty of Agriculture, Goce Delcev University-Stip, Republic of Macedonia during the period 2010-2015. The plant material used for isolation of the initial explants, was sterilized in the following manner: rinsing with tap water, than with distilled water, followed by 15-20 seconds in 70% C_2H_5OH, Tween 20 enriched, 10-15 minutes in 5% $Ca(ClO)_2$ enriched with Tween 80, and on the end the explants were rinsed few times with sterile water. Sterilized initial explants were cultivated on MS medium in which various concentrations and combinations of plant hormones were added.

Plant growth medium ingredients

In the following tables is shown that all plant explants except pepper anthers were cultivated on either on MS (Murashige and Skoog, 1962) or LS (Linsmaer and Skoog, 1965) media containing 3% sucrose, 0,7% agar, 100 $mg \cdot l^{-1}$ inositol, 200 $mg \cdot l^{-1}$ casein hydrolysate, 0,1 $mg \cdot l^{-1}$ B1, 1,0 $mg \cdot l^{-1}$ B6 and 0,5 $mg \cdot l^{-1}$ nicotinic acid. Different phytohormones were used in a medium such as: IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (α-naphtaleneacetic acid), BAP (6-benzylaminopurine), BA (N6-benzyladenine), KIN kinetin (6-furfuryl aminopurine), ZEA zeatin (N^6-4 hydroxyl-3-methyl-trans-2butenyl anunopurine), 2iP (N^6-2-isopentyl adenine) and 2,4 D (2,4-dichlorophenoxy acetic acid).
In order to favorite the microtuberisation of potatoes the medium for cultivation the nodal explant form *Solanum tuberosum* L. was supplemented also with different concentrations of sucrose (30, 60 and 90g/l) (Anoop et al., 2009; Dieme et al., 2013)
The androgenetic potential of the pepper was examined on medium developed by Dumas de Valux et al. (1981) method for pepper anther culture.

Growth conditions

Apical buds, cotyledons and hypocotyls from the species under research originated from plant seeds germinated on basal media. All the explants and their subculture to an new medium supplemented by same or different plant hormone were cultivated in climate chamber with controlled conditions and temperature of 25±2ºC, photoperiodic of 16/8 light/dark, 50% relative humidity, and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity. The growth conditions for examination of morphogenetic potential in pepper anther culture and the ability for androgenesis and/or somatic embryogenesis was set up according the method of Dumas de Valux et al. (1981).

Results and discussion

Micropropagation of some species form *Apiaceae* family

The species from family *Apiaceae* are a well-known source of many important herbal products. A number of research studies focus on the biosynthesis of secondary metabolites in *in vitro* plant cultures. There are also numerous reports concerning the methods of plant micropropagation, especially using somatic embryogenesis (Ekiert, 2000). The results from experiment shown in Table 1, confirmed that these species have an excellent potential for micropropagatein. Two types of initial explants, apical buds and shoots, were used to set up a tissue culture from celery, carrot and parsley on six different growth mediums. The best potential for morphogenesis of all mediums have the cultures from *Apium graviolens* L.

Micropropagation of some species form *Brassicaceae* family

This family includes many economically important edible and industrial oilseed, vegetable, condiment, and fodder crop. It also includes the molecular plant model, such as *Arabidopsis thaliana* and a rich source of agronomic and economic traits in its highly diverse wild germplasm (Warwick, 2011). Therefore the knowledge about the ability of micropropagation of these economically important species is of great importance for plant biotechnology. In Table 2 are presented the results of morphogenetic potential research of *in vitro* culture of broccoli, cabbage and radish. The best potential for morphogenesis of all examined mediums have the cultures from *Brassica oleracea* var. *italica.*

Table 1. The effect of plant growth regulators on morphogenesis in tissue culture of some species from family *Apiaceae*

Species form fam. *Apiaceae*	Explant	Medium + mg·L^{-1} Growth Regulators	Results	Efficiency
Apium graviolens L.	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	+++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+++
		LS + 5 KIN	shoots	+++
	shots	MS + 1 NAA	roots	+++
		MS + 1 IAA	roots	+++
Daucus carota subsp. *sativus* L.	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3 KIN+2 IAA+2 IBA	shoots	++
		LS + 5 KIN	shoots	+++
	shots	MS + 1 NAA	roots	+++
Petroselinum crispum Mill.	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+
		LS + 5 KIN	shoots	+++
	shots	MS + 1 NAA	roots	+++

Table 2. The effect of plant growth regulators on morphogenesis in tissue culture of some species from family *Brassicaceae*

Species form fam. *Brassicaceae*	Explant	Medium + mg·L^{-1} Growth Regulators	Results	Efficiency
Brassica oleracea var. *italica*	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	+++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+++
		LS + 5 KIN	shoots	+++
	shots	MS + 1 IAA	roots	+++
Brassica oleracea var. *capitata* L.	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+
		LS + 5 KIN	shoots	+++
	shots	MS + 1 NAA	roots	+++
Raphanus sativus var. *radicola*	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+++
		LS + 5 KIN	shoots	++

	shots	MS + 1 NAA	roots	++

Micropropagation of some species form *Cucurbitacea* family

The *Cucurbitacea* is a remarkable family with great and proven economic, aesthetic, cultural, medicinal and botanical significance.

Table 3. The effect of plant growth regulators on morphogenesis in tissue culture of some species from family *Cucurbitaceae*

Species form fam. *Cucurbitaceae*	Explant	Medium + mg·L^{-1} Growth Regulators	Results	Efficiency
Cucumis sativus L.	apical buds	MS + 11.0 KIN + 3.5 IBA	shoots	+++
	hypocotyls	MS + 2.0 KIN	callus	+++
	1/3 cotyledons	MS + 6.5 BA+10.0 2,4 D	callus	+++
Cucurbita pepo var. *cylindrical*	apical buds	MS + 2 KIN + 0,4 NAA	shoots	++
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3KIN+2 IAA+2 IBA	shoots	+
		LS + 5KIN	shoots	+++

Table 4. The effect of plant growth regulators on morphogenesis in tissue culture of some species from family *Solanaceae*

Species form fam. *Solanaceae*	Explant	Medium + mg·L^{-1} Growth Regulators	Results	Efficiency
Capsicum annuum L.	apical buds	MS + 5.0 BAP + 0.5 NAA	callus	++
		MS + 10.0 BAP + 0.5 IAA	shoots	++
		MS +1.0ZEA	shoots	+
	anthers	CP + 0,01 KIN + 0,01 2,4D	incubation	+
		R_1 + 0,01 KIN	embryos	++
	hypocotyls 1/3 cotyledons	MS + 30.0 BAP + 1.0 IAA	callus	+
		MS + 10.0 BAP + 0.5 NAA	callus	++
		MS + 5.0 ZEA	callus	+++
		MS + 2.5 2iP	callus	+++
Lycopersicon esculentum Mill.	apical buds	MS + 6.0 BAP + 0.4 IBA	shoots	+++
		MS + 4.5 BAP + 0.3 IBAA	shoots	++
		MS + 4.5 KIN + 0.3 IAA	shoots	++
	hypocotyls 1/3 cotyledons	MS + 6.0 BAP + 0.4 IBA	Callus	+
		MS + 3.0 KIN + 0.1 IAA	Callus	++
		MS + 1.5 BAP + 0.1 IBA	Callus	+++
Lycopersicon esculentum Mill. var. *cerasiforme* (Dunal)	apical buds	MS + 2.5 BAP + 1.5 NAA	shoots	++
		MS + 2.0 2iP + 0.5 IAA	shoots	+
		MS + 2.0 BAP + 2.5 2,4 D	shoots	++
		MS + 0.5 KIN + 1.0 IAA	shoots	+++
	hypocotyls	MS + 2.0 2iP + 0.5 IAA	shoots	+
		MS + 2.0 BAP + 2.5 2,4 D	shoots	+
		MS + 2.5 BAP + 1.5 NAA	shoots	+
	1/3 cotyledons	MS + 0.5 KIN + 1.0 IAA	callus	++
		MS + 2.0 2iP + 0.5 IAA	callus	++
		MS + 2.0 BAP + 2.5 2,4 D	callus	+++
		MS + 2.5 BAP + 1.5 NAA	callus	+++
Solanum	sprouts	MS + 0.5 BAP + 1.0 NAA	shoots	++

tuberosum L.		MS + 2.0 BAP	shoots	++
		MS + 4.0 KIN	shoots	+++
	nodule	1BAP+0.5NAA+30g/L sucrose	tubers	++
		4BAP+2 NAA + 60g/L sucrose	tubers	+++
		6BAP+2 NAA + 90g/L sucrose	tubers	+++

The family *Cucurbitacea* along with the other two *Brassicaceae* and *Asteraceae* can be consider as a family of extraordinary importance to humans, as they follow cereals and legumes in their economic significance to human kind (Lebeda et. al., 2006). A good micropropagation protocol could reduce the cost of hybrid seed production, which can account for 30% of the total seedling cost. The commercial application of *in vitro* techniques in *Cucurbitaceous* taxa has been well demonstrated and the regeneration of plants has been reported from different types of tissue cultures (Ahmad and Anis, 2005). Our experimental work involved meristem and non-meristem tissues cultivated on MS and LS media. The results show that *Cucumis sativus* L. possess bigger potential for morphogenesis as compared to *Cucurbita pepo* var. *cylindrical* (Table 3).

Micropropagation of some species form family *Solanaceae* family

The *Solanaceae* family provides some of the world's most popular vegetables. The family ranges from annual and perennial herbs to vines, lianas, epiphytes, shrubs, and trees, and includes a number of important agricultural crops, medicinal plants, spices, weeds, and ornamentals. Many members of the family contain potent alkaloids, and some are highly toxic.

In the current research the meristematic apical buds and non-meristematic cotyledonary and hypocotyledonary segments were used as initial explants for research of regenerative potential and further morphogenesis of pepper in *in vitro* culture (Table 4). The androgenetic potential of the pepper and the ability for embryos induction in pepper anther culture were tested as well (Table 4). From all tested media and treatments, haploid embryo production was accomplished only by the method of Dumas de Valux et al. (1981).

As experimental material for tomato micropropagation, apical buds, cotyledons and hypocotyls were used (Table 4). Tomato possesses excellent potential for *in vitro* morphogenesis which was proven by our and in many other experiments (Bhatia et al., 2004; Chandra, 2013; Koleva Gudeva et al., 2006)

The results from micropropagation of *Solanum tuberosum* L. presented in the Table 4 are in line with results of Dieme et al. (2013) who found that media enriched with BAP, KIN and sucrose give better microtuber formation. Different researchers agreed that higher percent of sucrose in the medium has positive results on microtuberisation process and increases the number and quality of microtubers (Farran and Mingo-Castel, 2006; Motallebi-Azar and Kazemiani, 2012; Ahmed et al., 2013; Iqbal et al., 2014; Koleva Gudeva et al., 2014) which confirm our findings during this research.

Conclusion

Today, without the use of *in vitro* plant methodology, many sophisticated and complex processes at the molecular level cannot be imagined and implemented, which is one of the most important challenges of the XXI century. The mass production of many species is based on the use of tissue culture and micropropagation. Furthermore, the ability to produce disease free plant material transformed plant propagation to an emerging industry for commercial micropropagation. This industry will continue to expand, but expansion will not be completed without introduction of new improved protocols for successful morphogenesis in plant tissue culture for economically important species, as they were presented in these paper.

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Original scientific paper
10.7251/AGSY1505245D

ANALYSIS OF FERITILITY ON THE MERLOT CLONES (Vitis vinifera L.), BY APPLYING THE METHOD OF CORRELATION

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Abstract

In this research were included seven clones of the variety Merlot (R-12, R-18, R-3, 181, 184, 348, 346). Fertility of each clone is determined by elements: fertility shoots per vine (%),absolute coefficient (number of clusters per fertile shoot), mass of bunch, and the yield expressed of unit area or kg/m^2.The obtained results from this work were statistically processed using the SPSS statistical software package 19, from where it can be measure the frequency, the percentage share of data, measures of central tendency (median, standard deviation, dispersion, arithmetic mean), such as graphic representation of data (a histogram).The analysis showed than all clones had larger correlation coefficient or there exists a greater dependence between the mass of the cluster and yields. From all clones, only two of them (R18, 346) proved more than greater dependence or greater correlation coefficient between absolute coefficientand the percentage of fertility shoot. The Merlot clone 348 proved the highest correlation between the mass of the cluster and yields where correlation coefficient is 0.983 which is approximately the value of 1, which indicates a very high connectivity.

Key words:*clones, merlot, fertility,correlation coefficient*

Introduction

Successful viticulture is based on quality vine varieties that are well adapted to external conditions and contributing to the high economic efficiency. In viticulture there are numerous clones of the grape vines, produced by clone selection. Clone selection is long procedure consists from finding, tagging and detailed describing of the variety on which is registered positive characteristic of a significant property. Clones of some varieties are actually descendants received from the vine variety, in that there are certain positive characteristics, permanent and stable in the next descendants that will grow from year to year. (Chien L. Mark,2008). After numerous testing and research on the resulting clone, he still carries the name of the variety where it belongs, and despite the name has a certain code or additional name, which would differ from the rest. Basic goals of the modern clone selection is separating a new clones which have to be virus free, to own resistance to the burners, ashtrays, and of course grapes hold better technological properties in relation to the native variet. (M. J. Benz et al.,2006).

Merlot vine variety is very important for the Republic of Macedonia, suggested to produce premium red wines. Merlot population contains many variations, features and clones.(V.Dimovska et. al., 2011).

The clone selection of Merlot is the most represented in France, Italy, Spain. As regards of technological features, selected Merlot clones which have larger yield, clusters with bigger weight give lower quality of the wine in accordance to less productive Merlot clones.(T.Slavtcheva, P.Pourtchev, 2007).

In order of population, clones from one selection differentiate with better characteristics of the grape and the quality of produced wines. (Larry J. Bettiga, 2003). The clones also differentiate between themselves in certain characteristics (yield, weight of the cluster, sugar

content, total acids and anthocyanin), which is a result of the assortment specification and less on the impact of cultivation conditions. (Matthew W.F. et.al., 2007).
In this study are included seven clones of Merlot (R-12, R-18, R-3, 181, 184, 348, 346). The clones were planted in the period 2009/2011 at the Agricultural Institute in Skopje, introduced from France. The main goal of this work is to compare the relationship or correlation between the mass and yields to individual clones and the correlation between the absolute correlation coefficient and the percentage of fertile shoots per vine.

Material and method

As a material for testing were used seven clones from Merlot: R-12, R-18, R-3, 181, 184, 348, 346 separately have grown in vineyards in the period 2009/2011, by applying appropriate agro-technical and ampelo-technical measures. Clones are introduced as certificated virus free planting material in 2000 from France. The test was performed at the Agricultural Institute in Skopje. The process of cultivation was a fruit-wall with two legged Gio's way of pruning, distance of planting of 2.5m between the lines and 1.3m between the grapevines in line.The clones were cultivated in same agro-ecological conditions with application of regular agro-technical and ampelo-technical measures.The most important parameters analyzed in this research were agrobiological properties, including fertility of shoots, expressed in % of developed and % of fertility shoots and yield of clones expressed inkg/m^2.After appearance of the shoots and the appearance of the earliest blossoms is determined number of fertility tendrils. These indicators allow the determination of the coefficient of fertility. During the harvesting was determined the number of bunches per vine, i.e yield of grapes expressed in kg/m^2. The obtained results from this work are statistically processed using the statistical software package SPSS 19, where we have measure for the frequency, the percentage share of data, measures for central tendency (mode, median, standard deviation, dispersion, arithmetic mean) and graphic display of the data (histogram). For the analyzed properties is calculated the correlation coefficient. Dependent variables critical in our work are the yields, a mass, the absolute coefficient of fertility and the percentage of fertility shoots at seven clones of Merlot over a period between 2009 and 2011. Using the software package SPPS 19 is made table display of correlation between mass and yields at individual clones and the correlation between absolute coefficient of fertility and the percentage of fertily shoots at the clones of Merlot. Comparing by the Pearson's coefficient of correlation we can see which parameters have a higher connectivity at the relevant clone. If $\rho = 1$, it means that there is a perfect linear dependence and the growth of the one variable well the other variable is growing. Correlation coefficient ranging between 0.9 and 1 means a very high connectivity. If $\rho = -1$ means that there is a perfect linear dependence, but with a growing the one variable declines other variable.

Results and discussion

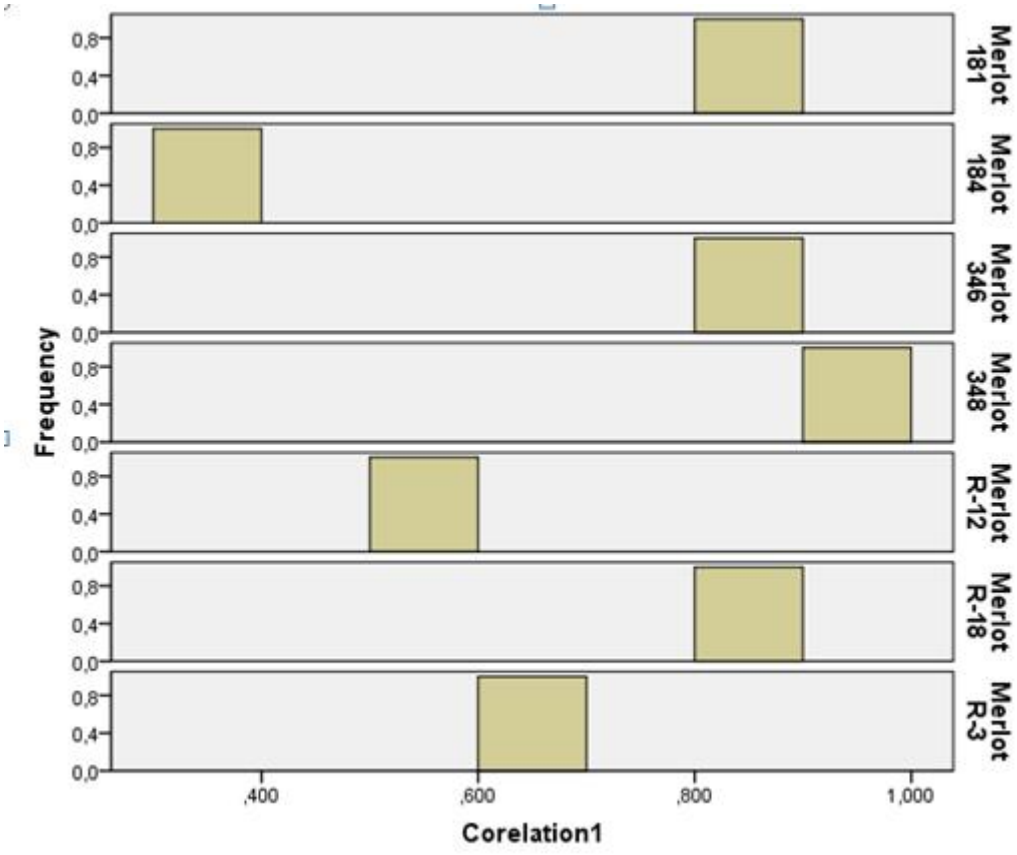
In Table 1. is detailed overview of the main elements of fertility of these seven clones of Merlot, for the period 2008-2010. For all elements is calculated the average value for these three years.Having into account this average value, we can note that in view of fertility shoots maximum value is occurs for the clone R-18,while a minimum value for the clone R-3. It is calculated the average value of the absolute coefficient, which shows again that the clone R-18 has the highest value, while the clone 346 minimum. According to Table 1.the average value for the mass of bunch shows the maximum value for the clone 184, while the minimum valuefor the clone 346. As an important element in the correlation yield expressed in kg / m^2 is included. The maximum value of the yield, refers to the clone R-18 and R-3, while a minimum value for the clone 181.

Table 1. Elements of fertility

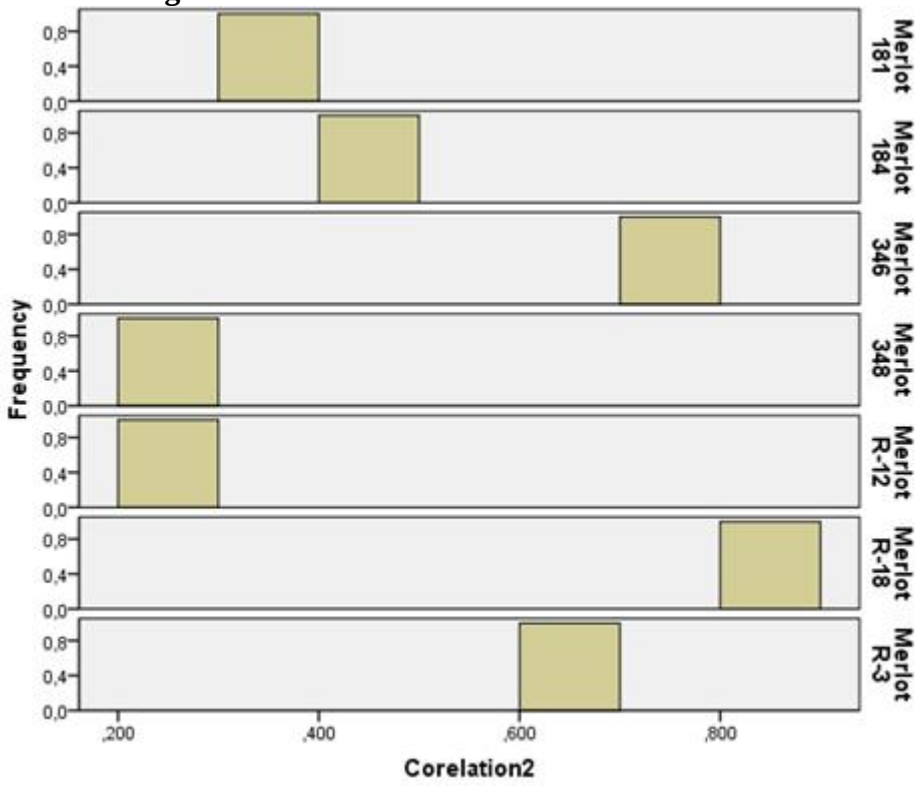
Clone	Year	Fertile shoots per vine (%)	Number of bunches per fertile shoot (absolute coefficient)	Mass of cluster (g)	Yield (kg/m^2)
181	2008	69.47	1.69	124	2.728
	2009	62.28	1.58	145	2.601
	2010	61.19	1.70	170	3.560
	08/10	**64.31**	**1.66**	**146.3**	**2.96**
184	2008	80.09	1.69	149	3.020
	2009	60.58	1.60	170	2.221
	2010	72.19	1.86	160	4.426
	08/10	**70.95**	**1.72**	**159.6**	**3.22**
346	2008	74.12	1.36	98	2.270
	2009	56.67	1.63	95	1.615
	2010	66.28	1.60	122	2.565
	08/10	**65.69**	**1.53**	**105**	**2.15**
348	2008	82.94	1.67	158	3.296
	2009	65.58	1.58	113	2.147
	2010	69.30	1.76	175	4.200
	08/10	**72.61**	**1.67**	**148.6**	**3.21**
R-12	2008	76.92	1.82	118	3.200
	2009	72.25	1.62	133	3.465
	2010	61.18	1.69	144	2.767
	08/10	**70.12**	**1.71**	**131.6**	**3.14**
R-18	2008	84.61	2.19	123	3.688
	2009	70.00	1.60	138	3.045
	2010	65.29	1.79	120	3.438
	08/10	**73.30**	**1.86**	**127**	**3.39**
R-3	2008	73.23	1.98	131	3.407
	2009	57.00	1.45	140	2.752
	2010	58.29	1.88	155	4.025
	08/10	**62.84**	**1.77**	**142**	**3.39**

Table 2. Display of Pearson's coefficient of correlation received from corelation 1 (mass *(g)*and yield*(kg/m^2)*) and correlation 2 (fertile shoots per vine *(%)* and absolute coefficient)

Clones	Corelation 1	Corelation 2
181	,868	,321
184	,332	,441
346	,804	,773
348	,983	,203
R-12	,542	,287
R-18	,851	,849
R-3	,605	,699



Graph. 1. The histogram shows correlation coefficient between the mass and yields



Graph. 2. The histogram shows correlation coefficient between the absolute coefficient and fertile shoots per vine

The first histogram shows that the Merlot 348 have the highest value of the correlation coefficient between the mass and yields while Merlo 184 lowest value. At the next histogram

indicates the correlation coefficient between the absolute coefficient and the percentage of fertility shoots to individual clones. The Merlot R-18 has the highest value of the correlation coefficient and Merlot and 348 and R-12 lowest value.

Conclusion

Under this research can be observed that for all seven shoots of Merlot higher correlation coefficient or greater dependence exists between the mass of the bunch and yields, unlike the percentage of fertily shoots and absolute coefficient of fertility.

From a total of seven shoots of Merlot, only two of them showed greater dependence, or greater correlation coefficient have absolute coefficient of fertility with the percentage of fertility shoots, and the values are close to the correlation coefficient of the mass and yields. In most of the cases it was proven that the correlation between the mass of bunch and the yield is higher, i.e. approximately equal for most clones of Merlot.

For the Merlot clone 348 has proved the highest correlation between the mass of bunch and the yield relative to other clones, where the Pearson's correlation coefficient is 0.983 which is approximately the value 1, indicating a very high connectivity.

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Original scientific paper
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MINERAL CONTENT OF GREEN VEGETABLES COMMONLY USED IN MONTENEGRIAN NUTRITION

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Abstract

Consumption of minerals is very important in human nutrition. Some play essential role in cell functions, while some are important in body maintenance and enzyme reactions. Healthy diet means getting enough of the essential mineral ingredients the body needs during the day. Minerals are inorganic substances and are found in the soil or in the water which is absorbed by plants. Montenegro as a Mediterranean country recommends in nutrition habits consumption of different kind of green vegetables. The aim of this paper was to determine the mineral content of some leafy vegetables such as spinach (*Spinacia oleracea)*, swiss chard (*Beta vulgaris)*, collard greens (*Crambe maritima*) and parsley (*Petroselinum crispum*) as a spice. Vegetable samples were collected from the private property "Green Home Jovović" during May 2015. 1g of washed, grounded and air dried sample was acid digested. Content of calcium, magnesium, potassium, copper, zinc and phosphorus was determined by ICP-OES. Based on the obtained data it can be concluded that Ca, Mg, K, Zn and P concentrations are the highest in spinach, 13.23 g/kg, 11.7 g/kg, 33.3 g/kg, 123.2 mg/kg and 5.4 g/kg, respectively, and spinach is a great source of Ca, Mg, K, Zn and P in daily diet. The lowest concentration of minerals is observed in parsley. As a good source of copper can be used collard greens, because it contains 93.0 mgCu/kg. The results showed that these values may be useful for the evaluation of dietary information.

Key words: *minerals, vegetables, human nutrition, diet*

Introduction

Agriculture, besides enhancing food production, is aimed to provide almost all the essential minerals and organic nutrients to maintenance a human health. Green vegetables, especially the ones that have been considered as functional foods, constitute the main source of minerals in the human diet (Acho et al., 2014; Martınez-Ballesta et al., 2010; Hanif et al., 2006). In Montenegro, production of vegetables increases because of better technical equipment and an improved technological level of production and use of high yield species and hybrids. The soil and climatic conditions of Montenegro are congenial for the production of vegetables such as swiss chard, spinach, collard greens and parsley as a spice (Borah et al., 2009; http://ec.europa.eu/agriculture/analysis/external/applicant/montenegro_en.pdf Accessed on 05/06/2015). These green leafy vegetables are inexpensive, are easily and quickly cooked, and constitute the main source of minerals in the human diet (Amaghol et al., 2012; Hanif et al., 2006; Rahmatollah et al., 2010).

Humans need inorganic nutrients, some of them are required in large amounts such as N, P, Ca, Mg, K, but others, are required in trace amounts (Fe, Zn, Cu, I ,Se) because higher concentrations can be harmful. Epidemiologically studies showed that optimal intake of elements, such as sodium, potassium, magnesium, calcium, manganese, copper, zinc, and iodine, could prevent disease such as cardiovascular disorders. There is a growing interest in the dietary minerals even though they comprise only 4–6 % of the human body. The major

minerals serve as structural components of tissues and function in cellular and basal metabolism and water and acid–base balance. The aim of this paper was to determine the mineral content of some leafy vegetables such as spinach (*Spinacia oleracea)*, swiss chard (*Beta vulgaris)*, collard greens (*Crambe maritima*) and parsley (*Petroselinum crispum*) cultivated in Montenegro. (Acho et al., 2014; Martınez-Ballesta et al., 2010; Rahmatollah et al., 2010).

Material and methods

For the purposes of this study content of minerals and nutrients have been analyzed in green vegetables that are the most consumed in Montenegro, as follows: Swiss chard, Spinach, Collard greens and parsley, as a spice. Vegetable were collected from private greenhouse Jovovic, near Podgorica, Montenegro. Sampling was carried on May 2015. It was analysed only the green part of the plant. All the products were washed with de-ionized water to remove the dust and soil particles and the fertilizers and spray residues and dried at room temperature. The samples were homogenisied until the powder is gained. Contamination during this step is avoided by using stainles steel equipmnt. Dried samples (0.5 g) were prepared by microwave digestion (ETHOS1), using HNO_3 and H_2O_2, in accordance with the recommendations of the manufacturer (7ml of HNO3 65% and 1ml of H_2O_2). Total concentrations of Ca, K, Mg, N, P, Cu and Zn were determninated using ICP-OES (Spectro Arcos) instrument For preparation of all reagents and calibration standards were used chemicals high purity and ultra pure de-ionized water (TKAMilli Q-Ultra Pure Water System, Germany). The standards for the calibration curve were prepared by several dilutions of certified solution concentration of 1000 mg / l from producers (JTBaker). All the experiments were carried out in triplicate (Borah et al., 2009).

Results and Discussion

The minerals and nutrient concentrations for each vegetables are shown in Figure 1-4. The results of the analyses were established to give nutrient/minerals values per 1000 grams of used portion of dried weight. Recommended daily intake minerals for healthy adult are showed in Table 1(http://www.healthbosnia.com/ishrana/klinicka/rdd.htm Accessed on 05/06/2015; http://www.lenntech.com/recommended-daily-intake.htm Accessed on 05/06/2015).

Calcium content ranged from 1.34 g / kg to 13.23 g / kg, potassium 20.80g / kg to 33.30g / kg, 3.23g of magnesium / kg to 11.70g / kg, of nitrogen 42.90g / kg to 61.90g / kg of phosphorus from 3.02g / kg to 5.40g / kg, copper from 0.016mg / kg to 0.039mg / kg, zinc from 0.028mg / kg to 0.123mg / kg. The highest mineral content is visible in the spinach, and the lowest in collard greens. The Spanach is also located the largest amounts of zinc, while the lowest content of copper and zinc has been observed in the parsley.

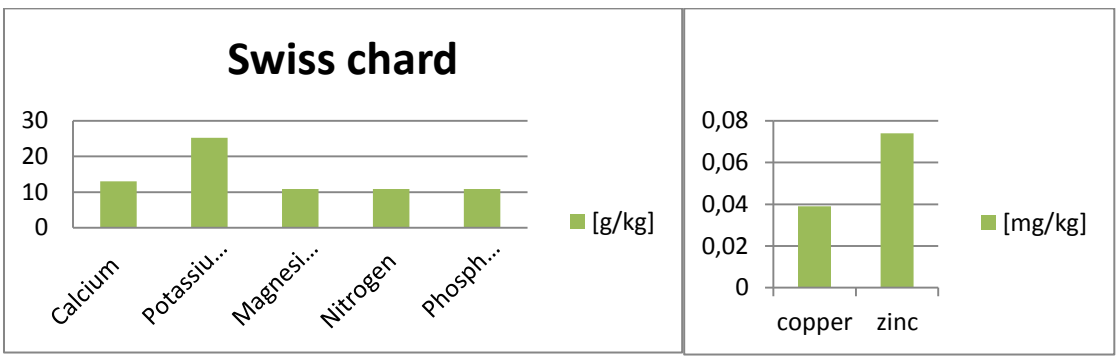

Figure 1. Minerals and nutrient concentrations in swiss chard

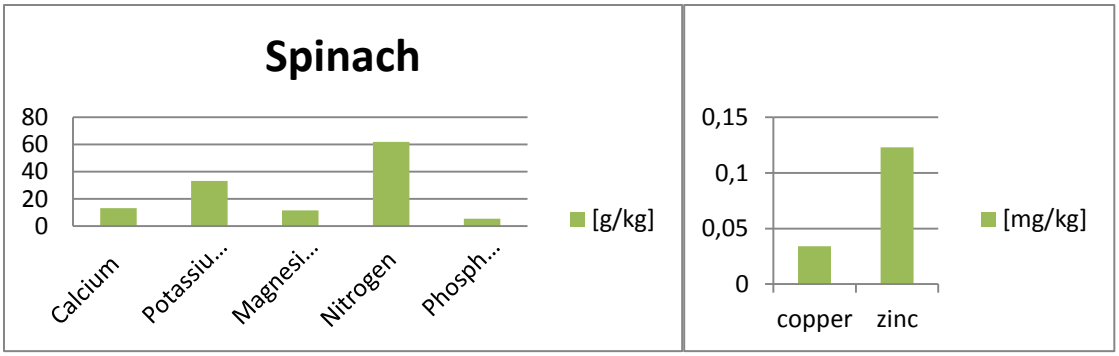

Figure 2. Minerals and nutrient concentrations in spinach

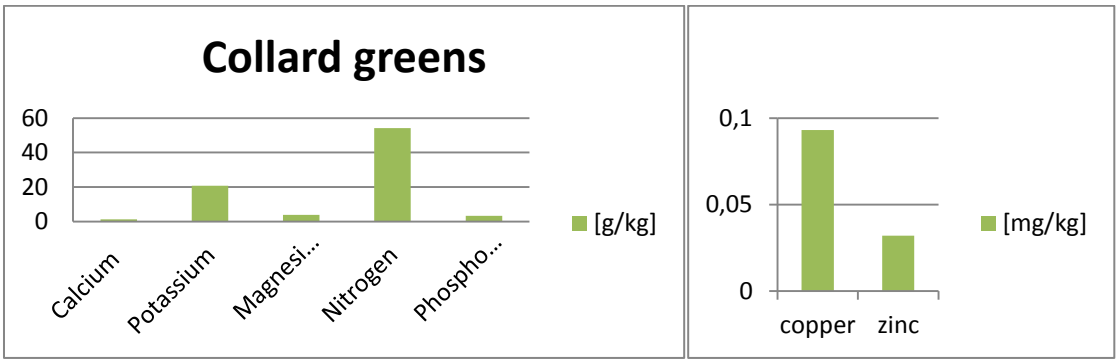

Figure 3. Minerals and nutrient concentrations in collard greens

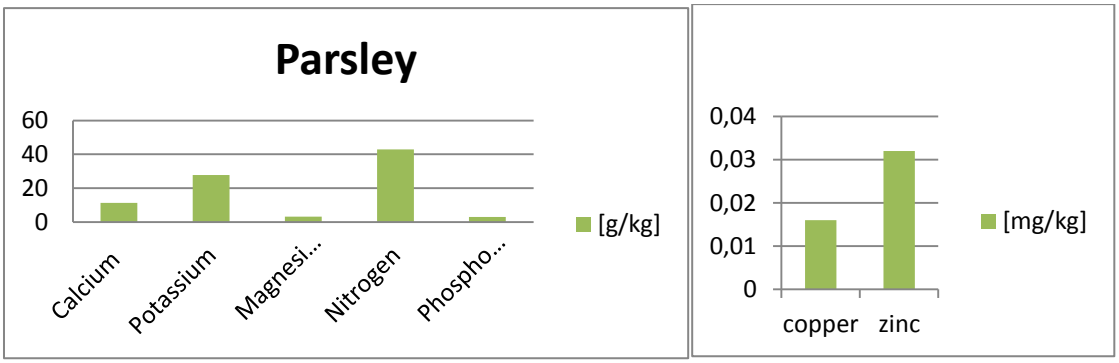

Figure 4. Minerals and nutrient concentrations in parsley

Table1: Recommended daily intake minerals for healthy adult)

Minerals	quantity (g)/ day	Over dosage
Ca	0,9	Doses larger than 1500 mg may cause stomach problems for sensitive individuals
K	2.0	Large doses may cause stomach upsets, intestinal problems or heart rhythm disorder
Mg	0.35	Doses larger than 400 mg may cause stomach problems and diarrhea

P	9.00	Larger doses may cause stomach problems for sensitive individuals
Cu	0.0009	As little as 10 mg of copper can have a toxic effect
Zn	0.013	Doses larger than 25 mg may cause anemia and copper deficiency

Conclusions

The results confirmed the well-known fact that green vegetables have an important role in the diet as a source of important nutrients and minerals. It was found that nutrients and minerals composition in all the selected vegetables was different. Some vegetables were rich in Mg and Ca such as s. chard and spinach but their concentration in other vegetables were poor especially parsley and collard greens. The results showed that these values may be useful for the evaluation of dietary information. The intake of these vegetables could be expected to contribute a large proportion of the mineral requirement in the body. It is recommended to use green vegetables in diets considering the facts that it is poor sources of fat that make them good food for obese people.

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Review paper
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USE OF PLANT BIOTECHNOLOGY IN ANTIOXIDANT PRODUCTION IN VEGETABLES

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Abstract

The interest in chemicals that act as antioxidants has increased during the last two decades because they are important in prevention of diseases which are a consequence of oxidative stress.

Antioxidants are chemically diverse group that prevent or slow the oxidation of other molecules by numerous mechanisms. They are very important in living world because they fight against free radicals. Based on a fact that food and health will always be getting priority for mankind, plant biotechnology techniques are employed for nutrition improvement of plants crops and production of antioxidants chemicals in vitro, for prevention of diseases. The main approaches of plant biotechnology for antioxidants production are plant genetic/metabolic engineering and plant tissue culture. The aim of this paper is to introduce some of the most potent exogenous antioxidants molecules for human health and how plant biotechnology can improve their content in commonly used crops. Also, this paper presents plant cell cultures technology as a factory for antioxidant production. Since the nutrition and health promotion are always been a priority of human kind improvement of food quality, its enrichment with components that act on prevention of chronic diseases, could be achieved through genetic engineering and breeding transgenic plants. The development of modern plant biotechnology is directed to overcoming obstacles in the form of bioavailability of plant products which have antioxidant properties and other useful features through concept of functional foods and nutraceuticals.

Key words: *antioxidants, plant biotechnology, genetic engineering, plant tissue culture*

Introduction

The interest in chemicals that act as antioxidants has increased during the last two decades because they are important in prevention of diseases which are a consequence of oxidative stress (Uttara et al., 2009). The state referred as oxidative stress is consequence of reactive species that are known as free radicals and can lead to numerous chronic diseases such as cardiovascular, inflammatory disease, cancer, aging and neuro-degenerative disorders (Alzheimer's and Parkinson's disease) (Djordjevic et al., 2008; Droge, 2002; Kunwar et al., 2011; Lobo et al., 2010). The most important free radicals molecules are reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are formed in cell during normal cell activities and in pathological states (Djordjevic et al., 2008; Valko et al., 2007. Antioxidants are chemically diverse group that prevent or slow the oxidation of other molecules by numerous mechanisms. They fight against free radicals and prevent the harmful effect on macromolecules (proteins, lipids and DNA). The harmful effects of ROS/RNS are balanced by the antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes (Fang et al., 2002; Lobo et al., 2010; Valko et al., 2007). Plant biotechnology techniques are employed for nutrition improvement of plants crops and production of antioxidants chemicals *in vitro*, for prevention of diseases. The main approaches of plant

biotechnology for antioxidants production are plant genetic/metabolic engineering and plant tissue culture (Kirakosyan et al., 2009).
The aim of this paper is to introduce some of the most potent exogenous antioxidants molecules for human health and how plant biotechnology can improve their content in commonly used crops. Also, this paper presents plant cell cultures technology as a plant factory for antioxidant production.

Plant genetic/metabolic engineering

In order to improve plant corps or introduce of novel products, in plants have been incorporated foreign gene so the transgenic plant is generated. Plants transformations is process in which genes are incorporated in organism's genome for amelioration of some plants traits. Figure 1 represents the most common used strategy in the formation of transgenic plants which is *Agrobacterium tumefaciens*-mediated transfer bipolar vectors into plant cells (Kirakosyan et al., 2009; Madigan et al., 2006).

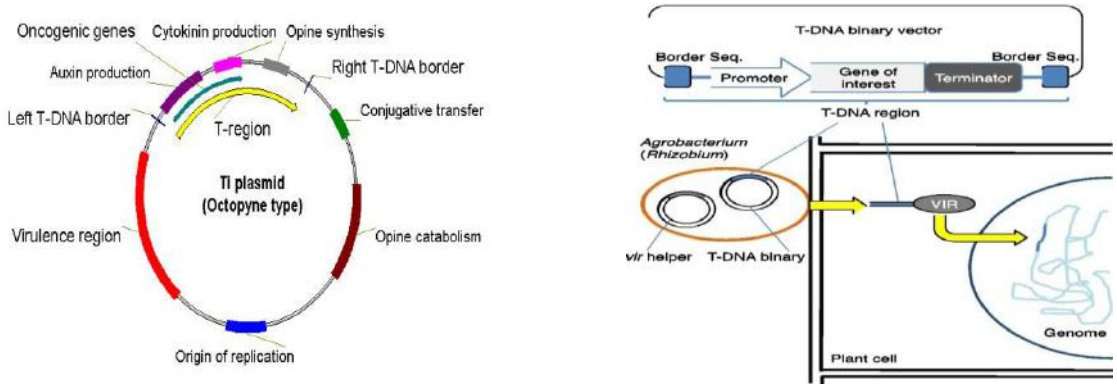

Figure1: Detailed view of Ti plasmid components; Incorporation of binary vector into plant cell for *A. tumefaciens*-mediated transfer

Genetic/metabolic engineering of flavonoid and carotenoids compounds

Flavonoids are class of phenolic compounds which perform a wide range of functions, such as antioxidant activity, UV-light protection and defense against phytopathogens (e.g. soflavonoids), legume nodulation, male fertility, visual signals and control of auxin transport (Balasundram et al., 2006; Ververidis et al., 2007).
Carotenoids are the pigments that give fruits and vegetables such as carrots, cantaloupe, sweet potato, and kale their vibrant orange, yellow, and green colors. There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen) (Rao et al., 2007; Schoefs, 2002).
The strategies of genetic engineering can be described with three approaches: 1) increment endogenous synthesis flavonoids using structural and regulatory genes; 2) blocking flavonoid pathway by RNA interference technique; 3) production of novel flavonoids inserting new branches in pathway (Bovy et al., 2007).

Strategy of regulatory genes engineering

Regulatory gene AtMYB12, originally transcriptional activator in *Arabidopsis thaliana*, when it is engineered in tomato fruit, activates biosynthetic pathway of polyphenols, chlorogenic acid and flavonol. In transgenic tomato fruit, engineering of this regulatory gene directs tissue specific expression (in tomato fruit), in plants parts that normally doesn't produce any flavonoids (ectopic expression) (Luo et al., 2008; Sanghera et al., 2013; Bovy et al., 2007).

Engineering of carotenoids content is not well developed (and use of regulatory genes), as in the case of flavonoids, because transcription factors that are control the synthesis of isoprenoids are not known. What has been done in this field is the observation that the Or gene (regulatory gene), isolated from the cauliflower and incorporated into potatoes leads to 6 times higher content of carotenoids in transgenic potatoes (Lopez et al., 2008; Sanghera et al., 2013; Lu et al., 2006).

Engineering of structural genes

In tomato fruit peel it is possible to detect some flavonoids while in the flesh all flavonoid genes show very low expression levels. In order to increase flavonoid level in tomato flesh, the four flavonoid biosynthetic genes from *Petunia* (*chs*, *chi*, *f3h* and *fls*) assembled in tomato and result was increased levels of flavonols in both peel (primarily quercetin glycosides) and flesh (primarily kaempferol glycosides) (Muir et al., 2001; Bovy et al, 2007; Verhoeyen et al., 2002).

To increase carotenoid content in plant crops, structural genes are mainly used, such as a crt B gene (originally from the bacterium *Erwinia uredovora*), whose product is phytoene synthase. When crt B gene is over-expressed in the transgenic tomato, result are increased total carotenoid content of 2.4 times compared to the control (Fraser et al., 2002; Sanghera et al., 2013).

Blocking specific steps in biosynthesis using RNA interference technique

RNA which is complementary with RNA transcript of some gene, is called RNA interference (RNAi). As example of this strategy is tomato *chs*1 gene (encode for the first enzyme in flavonoid synthesis) inhibition and result was decreased activity of chalcone synthase, which finally resulted in 99% reduction of the total flavonoid levels relative to wild type (Schijlen et al., 2007; Bovy et al, 2007).

Potato contains low levels of carotenoids, mainly lutein and violaxanthin which do not have the activity of vitamin. It was managed to increase the level of β-carotene 38 times, and 4.5 times of total carotenoids by blocking *chy1* and *chy*2 genes. Those genes coding for the enzyme β-carotene hydroxylase which is along with ε cyclization of lycopene is a key regulatory step in carotenogenesis of potatoes. Silencing of β-carotene hydroxylase directs metabolic flux towards the formation of β-carotene and lead to dramatic increase in a compound that is a precursor of vitamin A (Diretto et al., 2007; Sanghera et al., 2013; Bovy et al, 2007).

Introducing a novel branches in flavonoid biosynthetic pathway

Stilbens are the most important novel compound that had been introduced in transgenic vegetable. Stilbens are phenolic compound with antioxidant properties, and trans reverstatol is a major compounds. Reverstatol synthesis is controlled by the key enzyme stilben synthase (StSy) that majority of plant doesn't naturally produce. The production of revarstatol should be possible if StSy gen is provided. Revarstatol synthesis in tomato could be achieved by engineering of StSy gen from grape. This enrichment in nutrient content and antioxidant improvement of transgene tomato fruits doesn't affect the content of other phenolic compounds that are normally present in tomato (Giovinazzo et al., 2005; Bovy et al, 2007).

Plant Tissue Culture

The culture of plant cells, tissues or organs can be used, among other, also as a factory for production of antioxidant compounds in specifically formulated nutrient enriched media. A variety of different types of plant tissue cultures can be generated for antioxidant production and the most common used are callus culture, cell suspension culture, and plant organ culture.

In most *in vitro* culture system, the accumulation of biomass and production of the desired products (secondary metabolites) requires various medium conditions. The media composition has to be optimized so it would allow transit from cell growth to a state of production of metabolites (Kirakosyan et al., 2009; Matkowski, 2008).

Production of flavonoid by plant tissue culture

The most important group of flavonoids is flavone C-glycosides, because they are rare in plants and very important therapeutic molecules for disorders related with oxidative stress. It was shown that callus culture of *Passiflora quadrangularis* increase production of flavones when is treated with UV-B irradiation. Antioxidant activities compare to untreated calluses is higher with an increase ranging from 28% to 76% (Antognoni et al., 2007; Matkowski, 2008). Production of anthocyanins was obtained from selected callus cultures of *Ajuga reptans* in darkness and under a light-dark cycle and from cell suspensions culture which could only be established in a light-dark cycle. Anthocyanins which were detected was tentatively identified as a glucosylated cyaniding but the cell suspensions culture was not stable and end up either in low production line or in a complete colorless line (Callebaut et al., 1990; Matkowski, 2008).

Carotenoid production by plant tissue culture

Carotenoid production was achieved by suspension cell culture of *Artemisia annua*. It was found that suspension cell culture of produce carotenoids (lutein) and quinones (Q9 and Q 10) when suspension is treated with 2,6-dimethyl-B-cyclodextrins (DIMEB). Compare to untreated samples of cell suspension, application of elicitors, enhance production up to 2,5-fold of carotenoids and quinones (Rizzello et al., 2014; Matkowski, 2008).

Conclusion

Nutrition and health promotion are always been a priority of humankind. Improving the quality of food, its enrichment with components that act on prevention of chronic diseases is achieved through genetic engineering and breeding transgenic plants. Techniques of plant tissue cultures were investigated through potentials of antioxidants compound to be use in medical purposes, i.e. therapeutic application. The development of modern plant biotechnology is directed to overcoming obstacles in the form of bioavailability of plant products which have antioxidant properties and other useful features through concept of functional foods and nutraceuticals.

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EFFECT OF MICROBIOLOGICAL AND TECHNOLOGICAL PARAMETERS ON MONTENEGRIN RED WINES QUALITY

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Abstract

The aim of this study was to determine the effects of three different commercial yeasts, two different lactic acid bacteria (LAB) as well as addition of oak chips and blocks on quality parameters of Vranac, Kratošija and Cabernet Sauvignon young wines produced in vintages 2012 and 2013. The basic quality parameters of grape must were determined, and a total of 84 wine samples were analysed on quality parameters (alcohol content, total dry extract, pH value, total polyphenols and total anthocyanins).

Addition of different commercial LAB did not significantly influenced wine quality parameters, while oak addition influenced sensory properties of all examined wines. Regarding to Vranac wine, the highest content of polyphenols and anthocyanins was achieved when alcoholic fermentation was inoculated with commercial yeast BM 4x4, while alcohol content and total extract didn’t vary among experimental wines. In Kratošija wines combination of BM 4x4 and ICV D21 with oak blocks showed good results. In Cabernet Sauvignon wines addition of oak chips combination showed good results in all wines, while control wine had significantly lower marks. Results obtained in this study indicate the important role of variety, climate and winemaking techniques in the accumulation of these compounds in wine.

Keywords: *wine, yeast, lactic acid bacteria, oak, polyphenols, anthocyanins, wine quality*

Introduction

Montenegro is country with favourable conditions for viticulture and winemaking, what is confirmed by long tradition of grapevine breeding, where autochthonous varieties (‘Vranac’, ‘Kratošija’, ‘Krstač’ and ‘Žižak’) prevail. Among them, Vranac variety represents about 70% and it is used for production of premium red wines. Vranac has dark red ruby colour, full body, fruity taste and pleasant astringency, potential for aging and maturating in oak. According to Maraš et al. (2000), Ulićević et al. (1966) and Pejović et al. (1988), 'Kratošija' is Montenegrin autochthonous grapevine variety, grown in Montenegro for centuries. Kratošija wine is characterized by an intense ruby-red color and aroma of red berry fruits and an extremely pleasant taste; beside autochthonous, big attention is given to international introduced grapevine varieties, at first place to Cabernet Sauvignon and to its agrobiological, technological and economic characteristics in agro-ecological conditions of sub-region Podgorica.

Diversity of wine quality is quite unique among the products and determined mainly by interaction between grapes, yeast and technology. There are hundreds of different commercially available wine yeast strains that potentially provide a means by which winemakers can tailor their wines for different consumer market segments. For red wine, where non-volatile components that influence colour and mouth feel are critical determinants of style (Mercurio et al., 2010), the role of yeasts in modulation of phenolics may be of equal importance. Wine phenolics are extracted from grape skins and seeds during fermentation, interact with insoluble cell wall material from the grapes (Bindon et al., 2010b), and undergo

a range of chemical transformations during maturation (McRae et al., 2012). Selection of yeast strain has been shown to impact on the concentration of anthocyanins (Monagas et al., 2007; Morata et al., 2006) and other phenolics (Barcenilla et al., 1989; Monagas et al., 2007; Sidari et al., 2001; Torrens et al., 2008) in finished wine.

The role of lactic acid bacteria in winemaking is ambivalent. After alcoholic fermentation, LAB converts L-malate into L-lactate by decarboxylation. The so called malolactic fermentation leads primarily to a biological deacidification, but also to flavour modifications and microbial stabilisation. It has therefore a positive effect on wine quality (Liu, 2002). Even though, malolactic fermentation can occur spontaneously, *Oenococcus oeni* is often used as bacterial starter culture because of its high tolerance against ethanol and acidity.

Wine ageing is a well-known winemaking practice for improving wine quality and organoleptic characteristics. This practice modifies wine composition due to the compounds extracted from the wood and to chemical reactions that take place when oxygen passes through the wood pores or staves. Ageing wines in wood is expensive and barrels take up a lot of space in the winery and their lifetime is not too long. For this reason new inexpensive techniques have been developed to simplify the ageing process, while ensuring that the wood-related volatiles are released into the wine and with sensory properties similar to wines aged in barrels. These alternatives include oak chips, shavings and larger pieces of oak (Hooper and Marks, 1992). These alternatives to oak ageing have been an extended practice in the USA, Australia and other countries, such as Chile, for several years, but it was not a legal practice in EU countries until October 2006 when the EU approved the use of wood chips in winemaking and the designation and presentation of wine so treated (Commission Regulation (EC), 2006). The regulation states that “the pieces of oak wood” must come exclusively from the *Quercus* genus.

The aim of this study was to determine the effects of three different commercial yeasts, two different lactic acid bacteria (LAB) as well as addition of oak chips and blocks on quality parameters of Vranac, Kratošija and Cabernet Sauvignon young wines produced in vintages 2012 and 2013.

Material and methods

The trial was carried out during the 2012 and 2013 growing seasons. Autochthonous grapevine varieties Vranac and Kratošija, as well as, introduced one Cabernet Sauvignon were planted in the commercial vineyard of the company “13. Jul Plantaže” in the Cemovsko field in sub-region Podgorica (Montenegro).

Grapes of varieties Vranac nad Kratošija were planted in2003, grafted onto Paulsen 1103 rootstock, trained to a modified single Guyot training system, rows spaced 2.6 m apart and with 0.7 m between plants in the row. The grapevines of Cabernet Sauvignon were planted in 2006 (clone VCR 8), grafted onto Paulsen 1103/107 rootstock and trained to a single Guyot training system. The vine had a between-row and within-row spacing of 2.60 m x 0.70 m. All standard agro-technical operations were applied and vineyards were in good and healthy condition.

Wines were produced on a microvinification scale in the experimental cellar at the company “13. Jul Plantaže”. At harvest, grapes from all examined varieties were harvested manually and transported to the experimental cellar. Alcoholic fermentation of all trial was performed in PVC barrels using traditional method. For the vinification of control wines, an average grape sample of both varieties was 100kg of grapes. For trials with yeast addition we used an average grape sample of both varieties as follows: 400 kg of Vranac nad Kratošija varieties and 320 kg of Cabernet sauvignon variety. Potassium metabisulfite, purchased from Agroterm KFT, Hungary was added; 8 g 100 kg^{-1} of grapes from both varieties. All enzyme, wine yeasts, lactic acid bacteria and yeast nutrients were obtained from Lallemand, Australia. Three commercial yeasts, dominantly used for production of red wines, were chosen to induce

alcoholic fermentation. Within both varieties commercial yeasts that were used are: Enoferm BDX, Lalvin BM4x4 and Lalvin ICV D21 (30 g hL^{-1}). Enzyme Lalvin EX-V for maceration (2 g 100 kg^{-1}) and yeast nutrient Go-ferm protect (30 g hL^{-1}) were added during vinification, while yeast nutrient Fermaid E (25 g hL-1) was added during fermentation of both varieties. After alcoholic fermentation; wines were racked and separated in three same quantities for malolactic fermentation (two with different commercial LAB and one without addition of LAB). Commercial lactic acid bacteria that were used are: Enoferm ALPHA and Lalvin VP41. After completion of malolactic fermentation wines were racked, again separated in three same quantities for three months aging with oak alternatives, medium toasted (chips, blocks and control) produced by Toneleria Nacional. Also potassium metabisulfite was added in amount depending of free SO_2 in analyzed wine samples.
For determination of basic wine chemical parameters: alcohol, total acidity, tartaric acid, pH value, total dry extract, total and free SO2 the reference methods of European Union (Commission regulation (EEC) No. 2676/90 determining, 1990) were used. Total polyphenols and anthocyanins content were determined by spectrophotometer. Total polyphenols were quantified by Folin-Ciocalteu index method (Compendium of international methods of wine and must analysis-OIV, 2014). The total anthocyanins were determined using the pH differential method Giusti and Wrolstad (2001).

Results and discusion

Grape quality

Quality parameters of examined grape varieties are shown in Table 1. Vranac variety is known as variety with high total anthocyanin content, what is confirmed in this research. In average, Kratošija accumulates high content of sugar; low anthocyanins content and high total acidity is its varietal characteristics. Cabernet Sauvignon variety confirmed its high polyphenolic potential; however the larger berry size of Vranac and Kratošija in comparison to Cabernet Sauvignon dilutes the content of polyphenols in grapes (Pajović et al., 2014). Based on achieved results it can be concluded that Cabernet Sauvignon variety achieve good results in agro-ecological conditions of sub-region Podgorica.

Table 1. Quality parameters of Vranac, Kratošija and Cabernet Sauvignon grapes

Grape variety	**Vranac**			**Kratošija**			**Cabernet Sauvignon**		
Vintage	**2012**	**2013**	**average**	**2012**	**2013**	**average**	**2012**	**2013**	**average**
Sugar (%)	18.8	21.2	20.0	21.5	22.6	22.1	22.6	22.6	22.6
Total acidity (g/L)	3.61	4.63	4.12	5.73	6.23	5.98	6.72	5.71	6.22
pH	3.70	3.70	3.70	3.60	3.59	3.59	3.39	3.54	3.47
Tartaric acid (g/L)	4.84	5.49	5.19	3.64	4.05	3.85	3.52	5.87	4.70
Anthocyanins (mg/L)	195.0	205.5	200.0	60.0	21.0	40.5	15.0	61.5	38.3
Total polyphenols (g/L)	0.12	0.93	0.53	0.49	0.45	0.47	0.35	0.87	0.61

Meteorological data

In vintage 2013 the average air temperature in June was 23.3 °C, while in July and August was 27.2 °C and 26.9 °C, respectively (*Boreas*, agrometeorological station). Comparing average air temperatures during grape ripening (June, July and August), in 2012 the temperature was lower for 2.08, 1.45 and 0.20, respectively. In this period the total amount of rainfalls in 2013 were 153 L/m² and didn't distinguish so much from the climate normal, but it was much higher comparing to 2012, when during the same time the amount of rainfalls was 46.5 L/m². Unfavourable period was in the third decade of August when during the four

rainy days fell 84 L/m² of rain, and it could affect the quality of the yield, in contrast to August 2012, when the extreme temperatures in the same month (0.5 mm of rainfall) caused dehydration and premature harvesting berries.

Table 2. Average monthly air temperature (°C) in 2012 and 2013

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
2012	4.9	3.0	13.3	14.5	19.9	27.3	30.4	29.7	23.9	18.2	13.3	5.6
2013	6.7	7.7	10.8	17.1	20.1	24.5	28.5	28.8	21.3	16.7	12.5	6.6

Table 3. The amount of rainfall (L/m^2) in 2012 and 2013

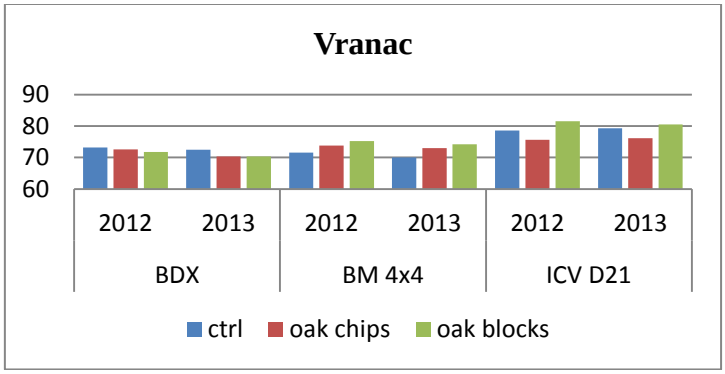
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
2012	60	201	0	351	132	34	11	1	87	296	286	305
2013	324	247	519	101	211	51	10	123	178	239	341	46

Wine quality

Quality parameters of examined wines are shown in Tables 4-6. Vranac achieved higher content of alcohol, total extract, total polyphenols and anthocyanins in 2013. The highest content of polyphenols and anthocyanins is achieved when alcoholic fermentation was inoculated with commercial yeast BM 4x4, while alcohol content and total extract didn't vary among experimental wines. Addition of different commercial LAB did not significantly influenced wine quality parameters among all examined wines. Oak addition influenced sensory properties of all examined wines (Graphs 1-3). Vranac wines showed potential for aging with oak chips and blocks, especially when alcoholic fermentation was inoculated with BM 4x4 and ICV D21.

Table 4. Wine quality parameters of Vranac variety

Quality parameter	**Alcohol (vol%)**			**Total extract (g/L)**			**Total polyphenols (g/L)**			**Anthocyanins (mg/L)**		
Vintage	**2012**	**2013**	**average**	**2012**	**2013**	**average**	**2012**	**2013**	**average**	**2012**	**2013**	**average**
CTRL	12.53	12.94	12.74	23.70	29.70	26.70	1.63	1.95	1.79	595.5	522.0	558.8
BDX	12.49	13.45	12.97	25.50	30.81	28.16	1.89	2.61	2.25	458.8	689.0	573.9
BM4X4	12.23	13.76	12.99	24.80	30.10	27.45	2.23	2.89	**2.56**	499.0	726.0	**612.5**
ICV D21	12.24	13.80	13.02	24.80	31.20	28.00	2.10	2.61	2.36	468.0	690.0	579.0

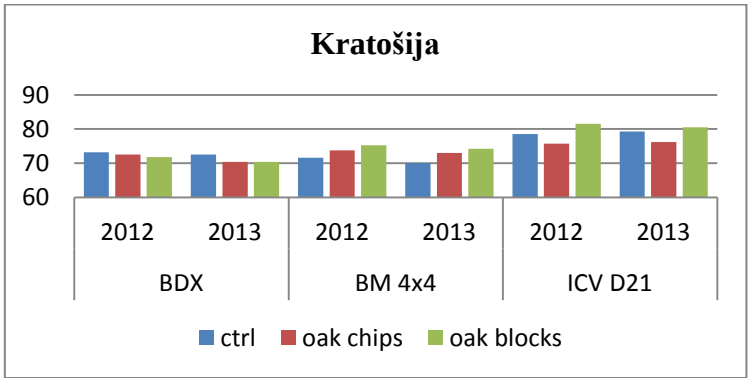


Graph 1. Sensory evaluation of Vranac wines

Kratošija wines quality parameters did not show significant differences among used yeast and LAB, while they influenced sensory properties of its wine. In Kratošija wines combination of BM 4x4 and ICV D21 with oak blocks showed good results, while when BDX was used control wines had better sensory evaluation.

Table 5. Wine quality parameters of Kratošija variety

Quality parameter	Alcohol (vol%)			Total extract (g/L)			Total polyphenols (g/L)			Anthocyanins (mg/L)		
Vintage	2012	2013	average	2012	2013	average	2012	2013	average	2012	2013	average
CTRL	13.44	12.81	13.13	24.20	27.04	25.62	1.28	1.33	1.31	132.0	222.0	177.0
BDX	13.81	12.91	13.36	26.50	27.98	27.24	1.94	1.69	1.82	143.5	260.4	202.0
BM4X4	13.70	13.02	13.36	26.00	21.01	23.51	1.76	1.65	1.71	148.0	209.0	178.5
ICV D21	13.86	12.90	13.38	25.40	27.04	26.22	1.64	2.01	1.83	189.0	214.0	201.5

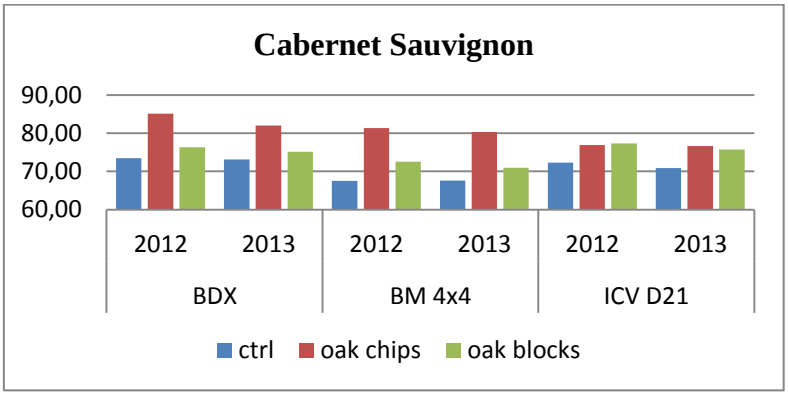


Graph 2. Sensory evaluation of Kratošija wines

Cabernet Sauvignon wines quality parameters also did not show significant differences among used yeast and LAB, while they influenced sensory properties of its wine. In Cabernet Sauvignon wines addition of oak chips combination showed good results in all wines, while control wine had significantly lower marks.

Table 5. Wine quality parameters of Cabernet Sauvignon variety

Quality parameter	Alcohol (vol%)			Total extract (g/L)			Total polyphenols (g/L)			Anthocyanins (mg/L)		
Vintage	**2012**	**2013**	**average**	**2012**	**2013**	**average**	**2012**	**2013**	**average**	**2012**	**2013**	**average**
CTRL	13.51	12.80	13.16	25.50	27.60	26.55	3.42	3.03	3.23	224.0	247.3	235.7
BDX	13.69	13.04	13.37	27.84	30.54	29.19	3.78	2.69	3.24	295.3	248.0	271.7
BM4X4	13.70	13.10	13.40	26.63	30.00	28.32	3.08	2.60	2.84	201.0	255.0	228.0
ICV D21	13.80	13.10	13.45	27.09	31.00	29.05	3.54	2.54	3.04	277.0	260.0	268.5



Graph 3. Sensory evaluation of Cabernet Sauvignon wines

Conclusion

This kind of research can be very useful to winegrowers and winemakers, in order to provide proper procedure for wine quality improvement. Regarding to Vranac wine, the highest content of polyphenols and anthocyanins was achieved when alcoholic fermentation was inoculated with commercial yeast BM 4x4, while alcohol content and total extract didn't vary among experimental wines. Addition of different commercial LAB did not significantly influenced wine quality parameters, while oak addition influenced sensory properties of all examined wines. Within Kratošija wines quality parameters it is noticed that there is no significant differences among used yeast and LAB, while they influenced sensory properties of its wine. In Kratošija wines combination of BM 4x4 and ICV D21 with oak blocks showed good results, while when BDX was used control wines had better sensory evaluation. Cabernet Sauvignon wines quality parameters also did not show significant differences among used yeast and LAB, while they influenced sensory properties of its wine. In Cabernet Sauvignon wines addition of oak chips combination showed good results in all wines, while control wine had significantly lower marks. Results obtained in this study indicate the important role of variety, climate and winemaking techniques in the accumulation of these compounds in wine.

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Original scientific paper
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COEFFICIENT SURFACE SPRAYED PLANTS: POTENTIAL TOOL OF EFFICIENCY PLANT SPRAYING

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Abstract

The objective of the paper was to evaluate the average coverage of sprayed plants depending on coefficient surface sprayed plants (W_{po}). Research was conducted in the laboratory at the Institute of Agricultural Engineering at the University of Life Sciences in Wroclaw (Poland) in 2015 years. The experiment was performed in three stages. In the first stage was defined the horizontal and vertical surface of the plants in order to determine the characteristics of the spray plant. To this was used specially constructed research position to which allowed taking pictures of the plants from all sides. The size of the surface projections is obtained by developing images in a graphics program AutoCad. The collected results allowed determining the coefficient of surface spray (W_{po}) adopted by the authors as a criterion that determines the number of spray characteristics of plants.

Then determined the degree of coverage of horizontal and vertical surfaces of plants. In research used four types sprayers: two standard and two ejector. Objects which were sprayed with artificial plants on which was mounted water sensitive papers. In order to determine the degree of covering, the papers were subjected to computer image analysis. Plants were sprayed at a constant operating speed (8 $km \cdot h^{-1}$) and the two pressures of 0,2 and 0,4 MPa. In the third stage of the research designated the average degree of coverage plants in the aspect of designated for him spraying the surface coefficient (W_{po}).

Analysis of the results led to the conclusion that the coefficient surface sprayed plants (characterized by the sprayed plant and its stage of development), may be helpful in selecting the right kind of sprayer to perform the surgery spraying plants.

Keywords: *average degree of coverage, sprayer type, spraying plants, the quality of the spray treatment*

Introduction

The quality of a spraying depends primarily on the degree of coverage of the liquid spray application as well as the uniformity of covering and the application of the liquid sprayed plants. The quality of a spraying depends primarily on the degree of coverage of the liquid spray application as well as the uniformity of covering and the application of the liquid sprayed plants. One of the deciding factors for obtaining the best quality, and thus the effectiveness of the treatments spraying is to choose the right type of surgery performed sprayer (Hołownicki et al., 2002; Lipiński et al., 2007; Godyń et al., 2008; Szewczyk et al., 2012). The indicators cannot fully assess the quality of surgery performed spraying, however allow compare used for surgery equipment and its accessories (types and kinds of nozzles).

Till now research showed that there is a correlation between the degree of coverage and the effectiveness of the biological treatment plant protection products. According to some scientists to provide plant protection against pests, degree of coverage of the plants through sprayer liquid should be at least 30% (Hołownicki et al., 2002). The research is not clear about what cover parts of plants going on and whether the value refers to the average degree of coverage calculated with reference to all components of the sprayed plants. In studies degree of coverage as samplers are most frequently used papers water sensitive. These samplers can be used for research in the field (Lipiński et al., 2007) and laboratory (Foque et

al., 2012). They are mounted directly to a lamina of the test plants. In the laboratory water sensitive papers are also trailed on artificial plants (Szewczyk et al., 2012). The advantage of artificial plants is always constant, the predetermined position of samplers in the space, that the results for research like this in authors opinion, are reproducible and comparable (Szewczyk, 2010).

Additional information in the assessment of a spraying plant may provide the user with spraying parameters such as - the average coverage of sprayed objects, and the spray characteristics of the plant, which defines the surface projections at different stages of development of the plant (Łuczycka et al., 2014).

Therefore, at the Institute of Agricultural Engineering at the University of Life Sciences in Wroclaw (Poland) we carried out the study whose aim was to determine the effect of the sprayed plants surface coefficient (W_{po}), on average cover plants taking into account the nature and type of sprayer used to spray treatment.

Materials and methods

Research was conducted in the laboratory at the Institute of Agricultural Engineering at the University of Life Sciences in Wroclaw (Poland) in 2015 years. The research was conducted in three stages. In the first step determined horizontal and vertical projected surface of plants, and then set the coefficient surface sprayed plants (W_{po}). The coefficient determined for the following crops: white beet in development 8 leaves and the initial phase of covering the spacing, rape in the phase formation of rosettes and shoot elongation phase, corn on the stages of development of the fifth leaf and the fifth node and potato in the early phase leaf growth and the creation of side branches.

Overall view and a schematic position of the spray characteristics of the test plants are shown in figure 1.

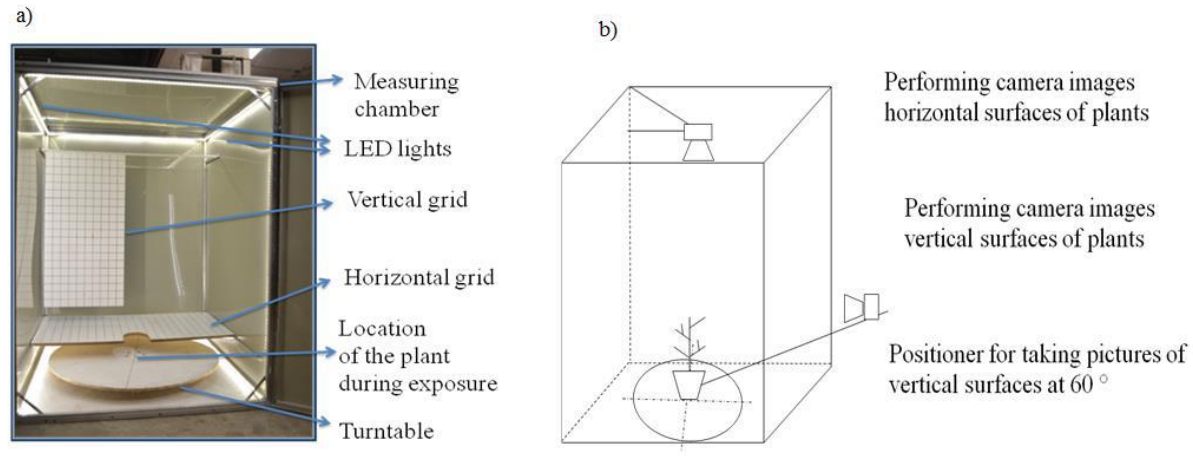

Fig.1 Position to study the characteristics of a plant spray a) general view, b) scheme (Source: own study)

Examined plants were placed into the measuring chamber on a turntable and rotating disc, every 60 degree were made pictures of the surface of vertical projections. In the horizontal surfaces camera placed over the center of the chamber and moved in a position up - down and front - back, depending on the size of the examined plants. The surface ratio of the examined plants was analyzed, so the distance between the camera and the plant did not make difference. The background of the surface plants was grid, where one side of the square had a dimension of 5 cm. As a result, with further analysis of photographs in a graphics program AutoCad read precisely the size of the surface projections of the test plants.

After reading the surface projections of plants, according to the formula 1 -calculated W_{po} for the various phases of development of plants. Surface values of vertical and horizontal projections of plants and W_{po} for the various phases of development are shown in table1.
The second stage of the research was to determine the degree of coverage of horizontal and vertical samplers. Samplers were water sensitive papers that were placed on artificial plants respectively on surfaces: horizontal (upper and lower) and vertical (inrun, departure, left and right).

$$W_{po} = \frac{projection\ vertical\ surface}{projection\ horizontal\ surface}\ [-]\ (1)$$

For testing selected four nozzles: standard (one-stream sprayers XR 11002 and two-stream sprayers DF 11002) and air induction sprayer (one-stream sprayers CVI 11002 and two-stream sprayers CVI TWIN 11002), and adopted the following operating parameters: a constant operating speed of the sprayer V =8 km·h^{-1}; various pressure: 0,2 and 0,4 MPa for which capability nozzles amounted to 0,65 l · min^{-1}and 0,91 l · min^{-1}; the height of the nozzles 0,5 m from sprayed objects. The scheme of test stand the degree of coverage shown in fig.2.

Table1. Characteristics of the spray plants

Cultivated plants	Development phase	Characteristics of the spray plants		
		Projection vertical surface [cm²]	Projection horizontal surface [cm²]	W_{po} [-]
White beet	8leaves	415,99	174,64	2,38
	beginning covering spacing	1470,34	612,90	2,40
Rape	forming rosette	190,80	89,40	2,13
	elongation shoot	1734,43	981,90	1,77
Corn	the fifth leaf development	354,47	116,70	3,04
	development of the first node	470,92	163,20	2,89
Potato	beginning leaf growth	68,70	55,30	1,24
	creation of side branches	2100,86	1078,90	1,95

Sprayers during the study time on the strictly designated track driving, which consisted of three parts (figure 2): a - run line, b - a measurement line, c - ending line. On the measuring placed three artificial plants. Degree of coverage was determined using a computerized image analysis method (in Adobe Photoshop). On the water sensitive papers three random selected parts on an area of 1 cm² was marked, which was read value of the surface covered with liquid during spraying. Then the formula 2 calculated the degree of coverage.

$$P_{sp} = \frac{W_k \times 100}{W_p}\ [\%]\ (2)$$

where:
P_{sp} – the degree of coverage of tested objects [%],

W_k – surface covered with liquid [piksele],
W_p – surface samples from 1cm² [piksele].

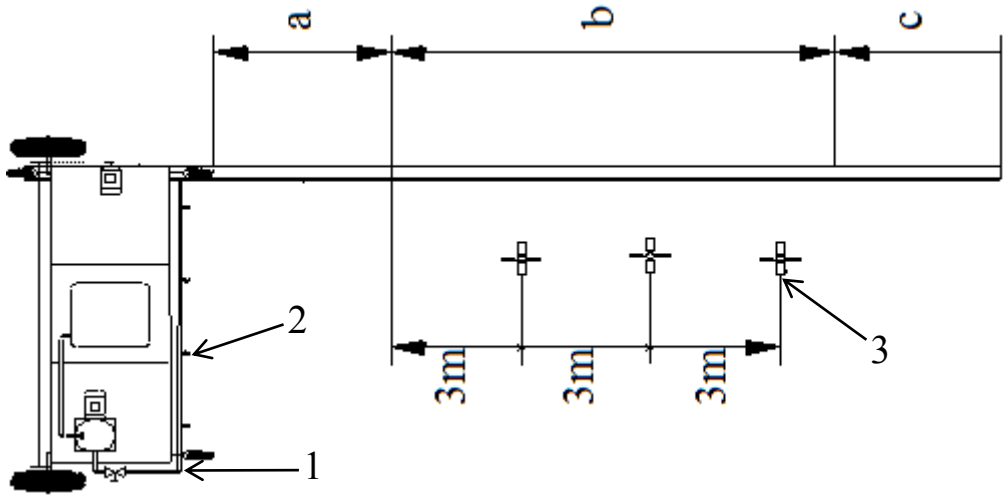

Fig.2. Schematic representation of the measurement stand: a – run line, b – a measurement line, c – ending line, 1 – sprayers carrier, 2 – nozzles, 3 – an artificial plant (Source: own study)

In the third stage of the research on the basis of the formula 3 determined the average coverage of sprayed objects.

$$P_{śr} = \frac{P_c}{\sum \text{projections vertical and horizontal surfaces}} \times 100\ [\%] \quad (3)$$

where:
$P_{śr}$ – the average coverage of sprayed objects [%], P_c – cover complete [cm²].
Complete cover (P_c) is the sum of those parts of the vertical and horizontal surfaces plants which have been covered with liquid during a spraying.

Results and discussion

The results of the average coverage for the test phase of development of individual plants and specific surface coefficient spray nozzles for pressure of 0,2 MPa is shown in figure 3, while the pressure of 0,4 MPa in figure 4.

The highest values of the average coverage at a pressure of 0,2 MPa was recorded for standard sprayer XR, the smallest whereas for sprayer air induction (two-stream) CVI TWIN. When the best average coverage for higher pressure (0,4 MPa) obtained for a standard (two-stream) DF, the weakest for CVI TWIN sprayer. After increasing the pressure to 0,4 MPa has also been observed general increase in the average coverage. eg. for potato in the beginning stage of development leaves (W_{po}= 1,2), the average coverage increased by approx. 6%.

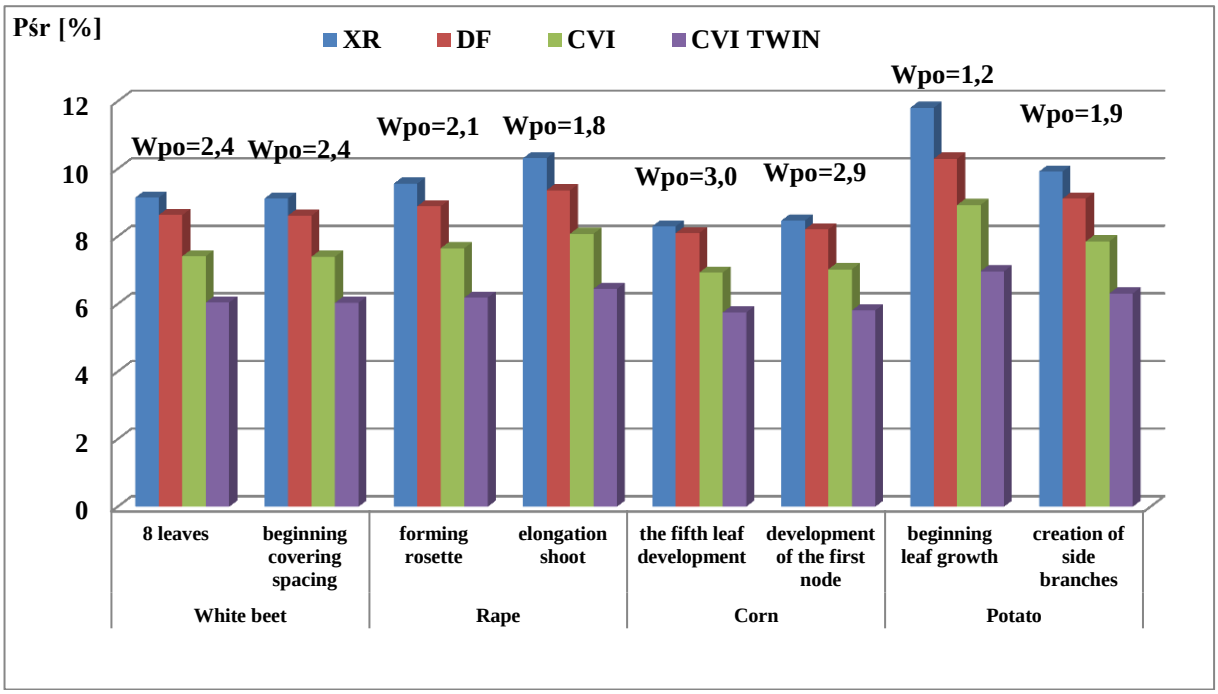

Fig. 3. Average coverage for selected phases of plant development and specific (W_{po}) tested spray nozzles at a pressure of 0,2 MPa (Source: own study)

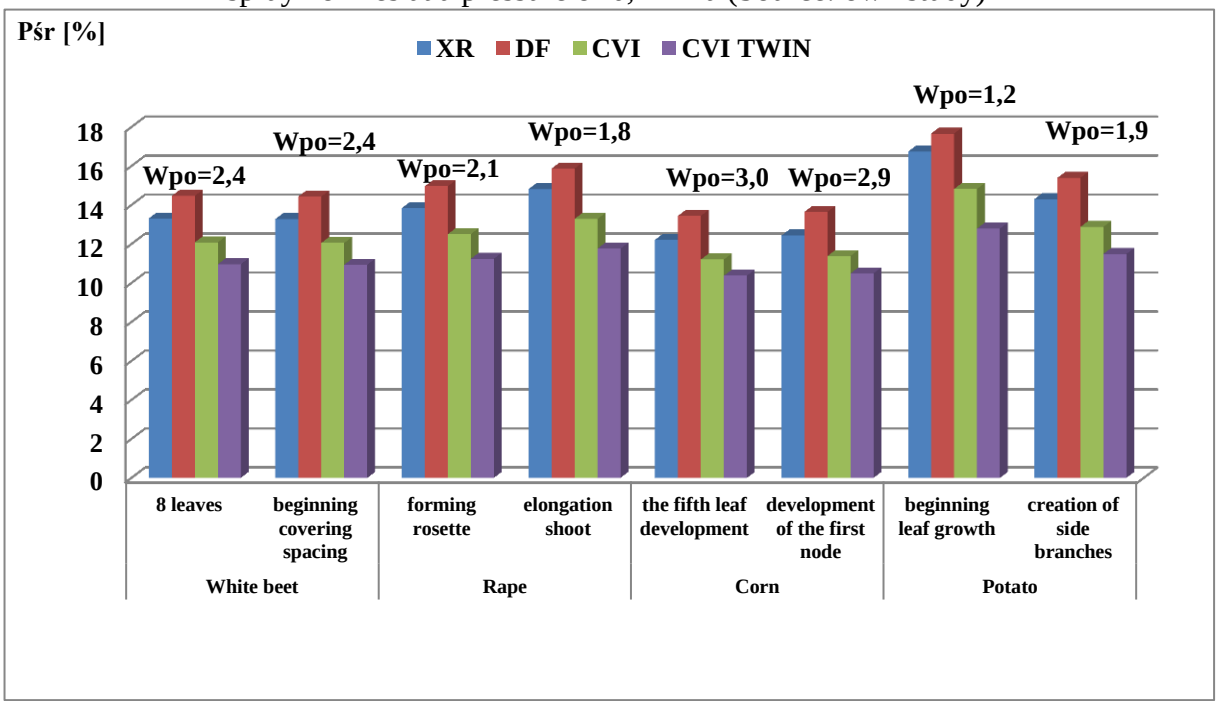

Fig. 4. Average coverage for selected phases of plant development and specific (W_{po}) tested spray nozzles at a pressure of 0,4 MPa (Source: own study)

The analysis graphs also show that the higher the value of the spray surface, (ie. the plant has more vertical surface), the lower the note value of average coverage. When the plant is characterized by a higher proportion of horizontal surfaces (lower values W_{po}), the higher the recorded value of average coverage of sprayed plants.

Conclusion

Based on the results obtained from the research and analysis the following conclusions: Adopted spray surface coefficient (W_{po}), characterized by the plant and its stage

of development have a decisive effect on the average coverage sprayed object.When the (W_{po}) obtained lower values (ie. the larger the surface area of the horizontal projection) is preferable to use spray nozzles standard, and when a definite advantage to vertical surfaces may be considered the use of standard dual stream nozzles, and in worse weather conditions ejector nozzles.

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Original scientific paper
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RESULTS REGARDING THE BIOMASS YIELD AT TRITICALE UNDER DIFFERENT TECHNOLOGICAL CONDITIONS

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Abstract

Triticale is a man-made crop obtained by crossing the wheat with rye. Among the characteristics that make this species of interest are the high yields of grains and biomass in a great diversity of climatic conditions and crop technologies with low inputs. The aim of the paper is to present the dry biomass yield and biomass moisture content at different varieties of triticale studied under different soil tillage conditions, different preceding crops, and different nitrogen application variants. In this respect, there were studied two varieties of triticale (Gorun 1 and Tulus) under the following technological conditions: two soil tillage, respectively ploughing and harrowing; two preceding crop, respectively sunflower and maize; six nitrogen application variants, respectively 0+0+0 kg.ha^{-1}, 40+40+40 kg.ha^{-1}, 40+80+0 kg.ha^{-1}, 0+40+80 kg.ha^{-1}, 0+80+40 kg.ha^{-1}, and 0+120+0 kg.ha^{-1}. Researches were performed in the agricultural year 2013-2014, in field experiments located in South Romania, respectively at Moara Domneasca Experimental Farm (44^{o}29’44’’ North latitude and 26^{o}15’28.5’’ East longitude) belonging to the University of Agronomic Sciences and Veterinary Medicine of Bucharest, on a reddish preluvosoil. The biomass determinations were performed at early dough growing stage. In the case of sunflower as preceding crop, the highest dry biomass yields were obtained for the nitrogen fertilization of type 40+40+40. For the soil tillage harrowing, when sunflower is the preceding crop for triticale, it was remarked also the nitrogen fertilization variant of type 40+80+0. In the case of maize as preceding crop and the soil tillage harrowing, the highest dry biomass yields were obtained for the nitrogen fertilization of type 0+120+0.

Key words: *triticale, dry biomass, soil tillage, preceding crop, nitrogen.*

Introduction

Triticale (*Triticosecale* Wittmack) is a man-made crop obtained by crossing the wheat with rye. It was designed in order to obtain a cereal which combines the good quality and high yielding capacity which is specific for the wheat with the tolerance to abiotic and biotic stress factors which is specific for the rye. Among the characteristics that make this species of interest are the high yields of grains and biomass in a great diversity of climatic conditions and crop technologies with low inputs. Triticale is able to resist some unfavourable biotic and abiotic environmental factors and thus produce good yield in marginal regions (Martinek et al., 2008; Lalević et al., 2012). Drought and frost tolerance are the primary advantages that triticale has over the other cereal crops and thus it reduces weather risk (Loha at al., 2007). In the specific growing conditions from South Romania this species is used for grain yields but also for producing biomass. The area from South Romania provides favourable growing conditions for triticale (Dumbrava et al., 2014). Triticale biomass was firstly of interest as fodder, but it has become also of interest as raw material for producing biogas (Ion et al., 2014). For biogas production, triticale should be harvested in the vegetation stage “grain in the milk stage” to “grain in the dough stage” (Amon et al., 2007). Understanding the

fertilization effect has been a continuous endeavour toward improving fertilization technology and strategy to reduce the negative impacts to increase the crop yield (Janušauskaitė, 2013). Nitrogen fertiliser application at different plant stages has an essential effect on the height of stems and grain yield quality (Alaru, 2004), and it is supposed to have an essential effect on the dry biomass yield. Also an essential effect on the dry biomass yield it is supposed to have the soil tillage and preceding crop.

The aim of the paper is to present the dry biomass yield and biomass moisture content at different varieties of triticale studied under different soil tillage conditions, different preceding crops, and different nitrogen application variants.

Materials and methods

Researches were performed in the agricultural year 2013/2014 in field experiments located in South Romania, these being implemented in the specific conditions from Moara Domneasca Experimental Farm (44°29'44'' North latitude and 26°15'28.5'' East longitude) belonging to the University of Agronomic Sciences and Veterinary Medicine of Bucharest. In the studied area, the specific soil is reddish preluvosoil. In this area, during the nine months of interest for triticale, respectively from September 2013 to June 2014, the average temperature was 9.5°C, while the multiannual average temperature is 8.5°C. The sum of rainfall was 572 mm, while the multiannual average rainfall is 408.9 mm. There were studied two varieties of triticale (Tulus and Gorun 1) under the following conditions: two soil tillage, respectively ploughing and harrowing; two preceding crops, respectively sunflower and maize; six nitrogen application variants, respectively 0+0+0 kg.ha^{-1}, 40+40+40 kg.ha^{-1}, 40+80+0 kg.ha^{-1}, 0+40+80 kg.ha^{-1}, 0+80+40 kg.ha^{-1}, and 0+120+0 kg.ha^{-1}. For the experimental variants having maize as preceding crop, we were not able to take valid samples of plants from the variants with soil tillage ploughing because of the high plant heterogeneity due to the poor and staggered plant emergence. For the variants with ploughing as soil tillage, one harrowing work was performed on 26th of September 2013, and the ploughing was performed at a depth of 18 cm two days later, on 28th of September 2013. For the variants with harrowing as soil tillage, two harrowing works were performed on 26th of September 2013, at a depth of 12 cm. Soil bed preparation was performed on 29th of October 2013, with a seed bed cultivator with two passages for the variants with ploughing and with one passage for the variants with harrowing. The sowing was performed in the same day as seed bed preparation, at 12.5 cm row spacing and at a density of 600 germinal seeds per square meter. Nitrogen applications were the following: first application in the autumn, before seed bed preparation (on 29th of October, 2013); second application in the spring, in the tillering growing stage (on 14th of March, 2014); third application in the spring, in the two nodes growing stage (on 26th of April, 2014). In the spring, the weed control was realised by applying the herbicide Dicopur Top 464 SL (344 g/l acid 2.4 D from SDMA + 120 g/l dicamba) at a rate of 1 l.ha^{-1}, the treatment being performed on 2nd of April 2014. The biomass determinations were performed at early dough growing stage. In this respect, the plants from 0.5 square meters, respectively four rows of plants on 1 m length were cut at soil level and were weighed immediately in the field. This procedure was performed in four replications for each experimental variant. For each experimental variant, ten average plants were taken and weighed immediately in the field and then dried in the laboratory into the oven at 80°C for 24 hours for determining the dry biomass and moisture content. The obtained data were statistically processed by analyses of variance. The yield of dry matter was calculated in tons.ha^{-1} and represents actually the yield of above-ground biomass.

Results and discussion

The highest dry biomass yield (expressed as above-ground biomass) was registered for Tulus variety (17.97 t.ha^{-1}) when the preceding crop was sunflower, the soil tillage was ploughing,

and the nitrogen fertilization was 0+40+80 (0 kg of N.ha^{-1} in autumn, 40 kg of N.ha^{-1} in the tillering growing stage, and 80 kg of N.ha^{-1} in the two nodes growing stage). But, for the Gorun 1 variety, the highest dry biomass yield (19.16 t.ha^{-1}) was registered when the preceding crop was maize, the soil tillage was harrowing, and the nitrogen fertilization was 0+120+0 (Figure 1). These data put into evidence the different reaction of the triticale variety to the technological measures. This idea is sustained also by the average values of the dry biomass yield at different nitrogen application variants. Thus, when the preceding crop was sunflower, the average dry biomass registered at different nitrogen application variants at Tulus was higher when the soil tillage was ploughing (15.45 t.ha^{-1}) compared to harrowing (13.32 t.ha^{-1}), while at Gorun 1 on the contrary the average dry biomass was higher when the soil tillage was harrowing (13.92 t.ha^{-1}) compared to ploughing (12.97 t.ha^{-1}).

In the variants with sunflower as preceding crop and when the soil tillage was ploughing, the highest dry biomass yields for Tulus variety was registered at nitrogen fertilization of 0+40+80 (17.97 t.ha^{-1}) and of 40+40+40 (17.60 t.ha^{-1}), in both cases with differences very significant compared to control represented by unfertilised variant (0+0+0). For Gorun 1 variety, the highest dry biomass yield was registered at nitrogen fertilization of 40+40+40 (16.04 t.ha^{-1}), with a difference significant compared to unfertilised variant (0+0+0). The high dry biomass yields registered at an application of nitrogen of 40+40+40 (autumn+early spring + later spring) put into evidence the importance of a well balanced nitrogen fertilization for triticale when the sunflower is the preceding crop and when the soil tillage is ploughing.

In the variants with sunflower as preceding crop and when the soil tillage was harrowing, the highest dry biomass yields for both triticale varieties (Tulus and Gorun 1) were registered at nitrogen fertilization of 40+80+0 (14.74 t.ha^{-1} for Tulus and 18.05 t.ha^{-1} for Gorun 1) and of 40+40+40 (14.56 t.ha^{-1} for Tulus and 16.83 t.ha^{-1} for Gorun 1). For both varieties and nitrogen fertilization variants the differences compared to unfertilised variant (0+0+0) were very significant. Compared to Gorun 1, at Tulus the difference very significant compared to unfertilised variant was registered also at nitrogen fertilization of 0+80+40 (15.64 t.ha^{-1}).

In the variants with maize as preceding crop and when the soil tillage was harrowing, the highest dry biomass yields for both triticale varieties were registered at nitrogen fertilization of 0+120+0 (14.54 t.ha^{-1} for Tulus and 19.16 t.ha^{-1} for Gorun 1), with differences very significant compared to unfertilised variant (0+0+0). This means that after maize as preceding crop, the highest dry biomass yields were registered when the nitrogen was applied in one application of 120 kg.ha^{-1}, early in spring (in the tillering growing stage), without nitrogen application in autumn or later in spring (in the two nodes growing stage).

The nitrogen fertilization variant of 0+40+80 determined the smallest dry biomass yields when the soil tillage was harrowing at both triticale varieties for sunflower as preceding crop (11.15 t.ha^{-1} for Tulus and 9.98 t.ha^{-1} for Gorun 1), and at Gorun 1 also for maize as preceding crop (13.76 t.ha^{-1}). These small dry biomass yields are determined by the lack of nitrogen in the autumn, less nitrogen applied early in spring (in the tillering growing stage) and the less efficient use of the high nitrogen rate applied later in spring (in the two nodes growing stage).

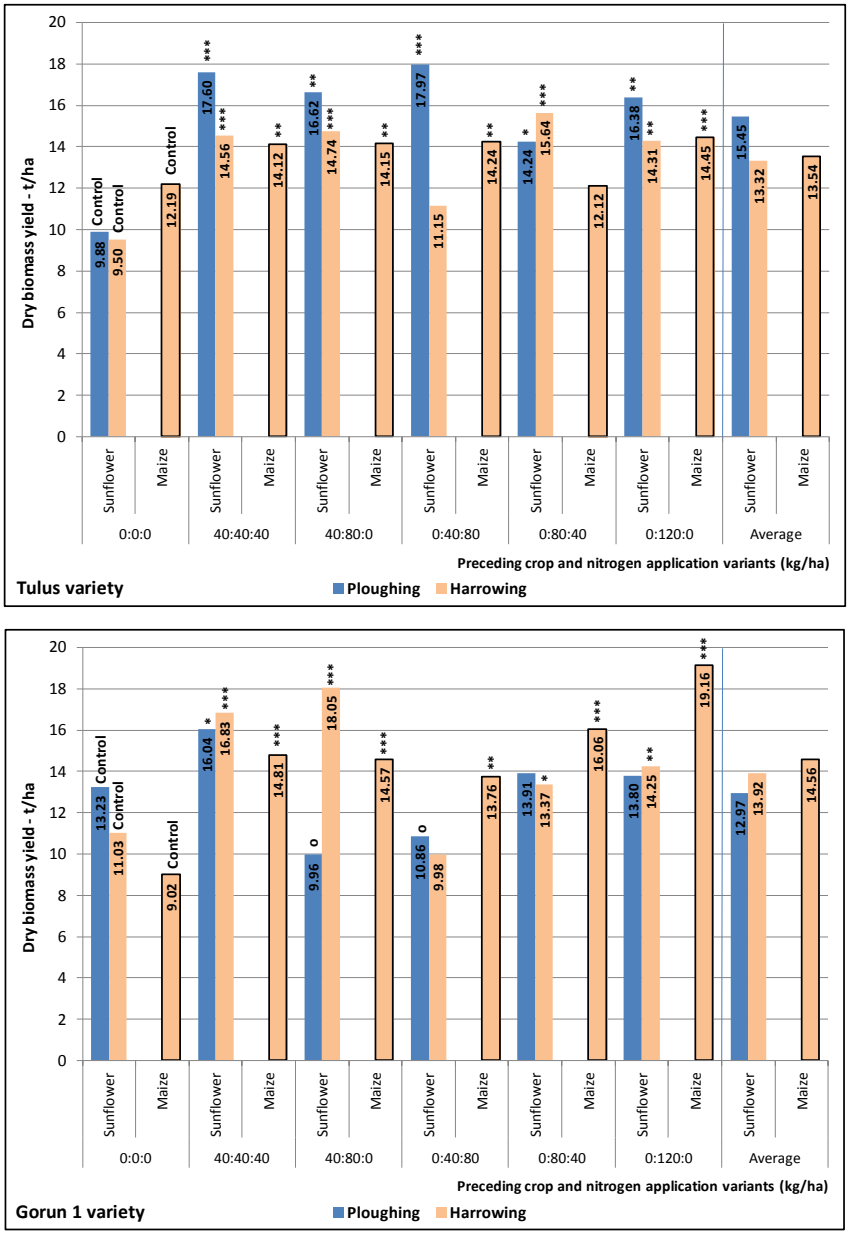

Figure 1. Dry biomass yield at Tulus and Gorun 1 triticale varieties under different soil tillage, preceding crops and nitrogen application variants

Nitrogen application of 0+40+80 with soil tillage harrowing and sunflower as preceding crop determined the highest values of the biomass moisture content for both studied varieties (Tulus and Gorun 1), respectively 49.42% for Tulus and 47.86% for Gorun 1. Also a high value of the biomass moisture content was registered under the same experimental conditions for maize as preceding crop, respectively 41.16% for Tulus and 40.85% for Gorun 1 (Figure 2). This is explained by the fact that the high nitrogen rate applied late in spring (in the two nodes growing stage) keeps the plants green, respectively the vegetative plant components.

The smallest values of the biomass moisture content (respectively the driest plants) were registered for both triticale varieties when the preceding crop was sunflower and when the soil tillage was ploughing, but at nitrogen application of 0+80+40 for Tulus variety (22.59%) and at nitrogen application of 40+40+40 for Gorun 1 variety (19.26%). It has to be emphasised that also for Gorun 1 variety the nitrogen application of 0+80+40 with soil tillage ploughing determined a small value of the biomass moisture content, respectively 22.74% (Figure 2).

When the preceding crop was sunflower, for both triticale varieties the average biomass moisture content was smaller when the soil tillage was ploughing than when the soil tillage was harrowing. When the soil tillage was harrowing, for both triticale varieties the average biomass moisture content was smaller when the preceding crop was maize than when the preceding crop was sunflower.

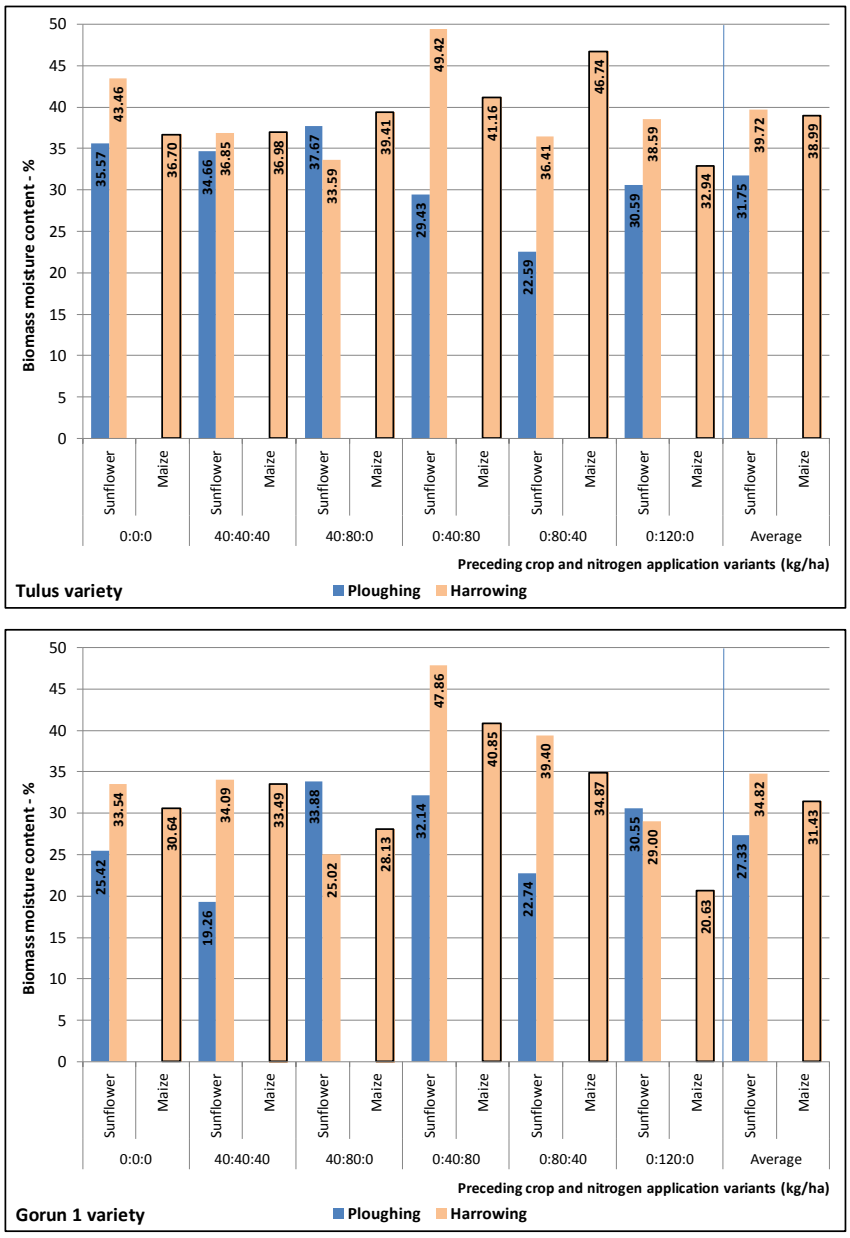

Figure 2. Biomass moisture content at Tulus and Gorun 1 triticale varieties under different soil tillage, preceding crops and nitrogen application variants

Conclusion

Taking into account the data we have obtained one can conclude that triticale varieties react different to the technological measures.

In the case of sunflower as preceding crop for triticale, the highest dry biomass yields were obtained for the nitrogen fertilization of type 40+40+40 (autumn + early spring + later spring) both for soil tillage ploughing and harrowing. For the soil tillage harrowing, when sunflower is the preceding crop for triticale, it was remarked also the nitrogen fertilization variant of type 40+80+0.

In the case of maize as preceding crop for triticale and the soil tillage harrowing, the highest dry biomass yields were obtained for the nitrogen fertilization of type 0+120+0.
In the case of soil tillage harrowing, the nitrogen fertilization variant of 0+40+80 determined the smallest dry biomass yields, this being a consequence of the lack of nitrogen in the autumn, less nitrogen applied early in spring (in the tillering growing stage), and less efficient use of the high nitrogen rate applied later in spring (in the two nodes growing stage).
Nitrogen fertilization variant of 0+40+80, with high nitrogen rate applied later in spring (in the two nodes growing stage), kept the plants green, which determined the highest values of the biomass moisture.
Nitrogen fertilization variant of 0+80+40, in the case of soil tillage ploughing and with sunflower as preceding crop determined the smallest values of the biomass moisture (respectively the driest plants).
In the case of sunflower as preceding crop, the average biomass moisture content was smaller when the soil tillage was ploughing than when the soil tillage was harrowing.
In the case of soil tillage harrowing, the average biomass moisture content was smaller when the preceding crop was maize than when the preceding crop was sunflower.

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MORPHOLOGICAL PLANT PROPERTIES OF SWEET CORN CULTIVATED WITH DIFFERENT TECHNOLOGIES

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Abstract

Aim of experiment set up in 2013, to study evoluation of sweet corn growing period with application of some technological elements: propagation time, propagation method, floating row cover. The choosen variety was a conventional sweet corn hybrid, very early ripening 'Spirit'. The following growing technologies were compared: 1. direct sowing of plants with floating row cover, early period 2. direct sowing of plants without row cover, early period 3. plants transplantation with floating row cover, normal period and 4. direct sowing of plants without row cover, normal period (regarded as control).

It was studied the influence of above mentioned technological elements on some important morphological properties of sweet corn plant: evolution of phenological phases, plant highness, tassels length, ear weight, highness of ear insertion.

Key words: *earliness, sweet corn, transplantation, fleece covering*.

Introduction

Based on its present growing area, the sweet corn is the vegetable which is grown on the greatest area in Hungary. After dates of Hungarian Fruit & Vegetable Interprofessional Organization in 2003 the growing area was about 38,000 hectares. After 2003 followed a suddenly and sharp decline. After diminishing, the plant returned in rise, in 2006 against over 30,000 hectares. The recession mentioned above affected not only Hungary but also the holdings of the USA and Western Europe. In the case of the former, however, the increase in fresh consumption partly counterbalanced the rate of decrease. Near 50% of world sweet corn production came from US (Slezák *et al.*, 2012). In 2013 the growing area was 34, 000 hectares, less with 1000 ha, compared to 2012 (Avar, 2013).

In order to promote fresh consumption, as well as to maintain and increase the sweet corn exports, it is necessary to promote investigations so as to be able to ensure a further increase in the growing area and yields of sweet corn with the help of the experiences. The literature mainly is concerned with maize growing technology, but a lot of dates could be used by sweet corn. Of the production technology elements, a number of researchers studied or are currently studying the sowing time of sweet corn.

As early as at the ending of 19^{th}- and the beginning of the 20^{th} century some researchers (Cserháti, 1901) highlighted the importance of the sowing date. Ripening can occur earlier when sowing earlier and using high quality seeds as compared to normal or late sowing. I'só (1969) and Pásztor (1966), after their multi-year sowing date trial, concluded the following: in the case of an earlier sowing seed germination will be more protracted, but silking and harvesting occur sooner than by lately sowing time. After multi-year trial Berzsenyi et al (1998) have studied the effect of different sowing times on maize development.

Several techniques are known in the art for the purpose of early fresh market shipments: seedling growing or direct seeding with temporary plant cover (Hodossi, 2004).

About the covered early sowing as a technological variation Aylswirth (1986) mentioned, that from an early sowed crop, made in first week of April, arranged in twin rows (42 cm) and covered by plastic, we could harvested marketable cobs by the fourth of July. In case of direct

sowing, as propagation method, another earliness increasing solution is the temporary covering with plastic or vlies, used in different combinations (Hodossi and Kovács, 1996).
The most widespread method of seedling production is the use of soil blocks (Pereczes, 1999) which can also significantly increase earliness. The combined application of seedling growing and floating row cover can advance harvest by three weeks as compared to the traditional technology and can give farmers a three to four times greater income (Kurucz, 1998). Rattin et al., (2006) compared direct sowed and transplanted sweet corn varieties, without covering and concluded no difference, in ear weight and ear length, between transplanted treatments plant's, in comparison to direct sowing treatments plant's.

Materials and methods

The experiment were set up in 2013 on an area equipped for irrigation at Carei, Satu Mare County, situated in NW part of Romania. Conventional, reliable and sufficiently known among growers sweet corn variety, Spirit, was used as a reference variety in the variety comparison trials of the Central Agricultural Office. Hybrid has short growing period of 85 days and yellow kernels. Average height of plants is 159 cm, average ear height is 37 cm, ear length 19.6 cm and average ear weight is 245 g. The variety was granted official recognitions in 1988 and has been the dominant variety of the early ripening category till now. In the year prior to the experiment the area was under wheat.
The following treatments, each with four replications, were applied during the experiment:
P1 = direct sowing of plants with floating row cover, early period (11^{th} April)
P2 = direct sowing of plants without row cover, early period (11^{th} April)
P3 = plants transplantation with floating row cover, normal period (21^{th} April)
P4 = direct sowing of plants without row cover, normal period, regarded as control (21^{th} April)
For the frame structure of the treatments with cover we used ø 4.2 mm zinc coated wire coils. The fleece, 60 cm in width, was stretched over a small tunnel of 40 cm in height and then its edges (25-25 cm, respectively) were covered with soil using a hoe and the its ends were tied to the stakes hammered down. The construction of the frame structure and the setting out of the fleece cover were carried out at the same day as direct seeding and out planting. For the purpose of seedling growing, the seeds were sown on 5^{th} April 2013, in trays with rigid walls having 3,7x4x5,4 cm size. For growing the seedlings, commercial mix made of white peat 10-20 mm, PG Mix 1 kg/m^3 + micro nutrients, bentonite 40 kg/m_3, pH 5,5-6,5 was used. The seedlings were planted out at the 3 to 4 leaf phenological stage. At the two propagation times the treatments P1 and P3 were covered with Novagryl floating row cover, having a weight of 19 g/m^2, (using the small tunnel technique) in order to enhance earliness. The stand was created to contain 60,607 plants per hectare, according to the recommendations of the owner of the variety, at a spacing of 110+40x22 cm in twin rows. Each plot had an area of 6x3,5m (8 parallel rows and 16 seeds sown in each row). Sowing depth was 3 cm. The edge was the respective outer rows of the 4 twin rows of the plot. In October 2012, 35 t/ha of farmyard manure was worked into the soil with ploughing on area. Nitrogen fertilizer (120 kg/ha) was applied at the 6-7 leaf stage, the form of top dressing. The fertilizer application was worked into the soil with a rotary hoe.
During the experiment, we studied plant growth rates and recorded the time of the occurrence of the major phenological stages. For this purpose, we carried out regular observations (every 3 to 5 days) according to the following:
beginning of seed emergence (appearance of first germs),
appearance of tassels (by 50% of the plants),
beginning of tasseling (pollen shed has begun on the axes of tassels),
50% silking (silks have reached a length of 2 cm on half of the ears) "milky stage" (harvest).

During the experiment, we studied some plant morphological properties according to the following:
highness of plants (cm),
length of tassels (cm),
highness of ear insertion (cm).
Ears, together with the husks, were collected from the four central (two twin) rows and 20 ears of average appearance were selected from each row for following measurement:
unhusked and husked ear weight (gram).
The statistical analysis of the results was carried out by using the programme *RopStat 1.1.* When the standard deviations were identical the mean values were compared by pairs using the *Tukey-Kramer* test, while in the case of the non identical standard deviations the means were compared using the *Games-Howell* test (Vargha, 2007).

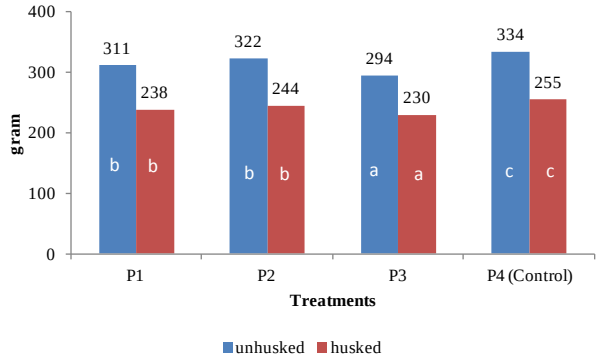
Results and discussion

Table 1. illustrates the times of sowing, emergence as well as the number of days elapsed from the date of direct seeding or transplanting to the different phenological stages.

Treatments (sowing day)	Date of emergence (days)	Tassels appearance by 50% (days)	Stigma appearance by 50% (days)	Starting harvest (days)
P1 (IV. 11)	17 days (IV. 28)	58 days (VI. 8)	63 days (VI. 13)	79 days (VII. 1)
P2 (IV. 11)	17 days (IV. 28)	62 days (VI. 12)	67 days (VI. 17)	82 days (VII. 3)
P3 (IV. 21)		34 days (V. 31)	40 days (VI. 6)	60 days (VI. 26)
P4 (control)	8 days (IV. 28)	57 days (VI. 17)	62 days (VI. 22)	81 days (VII. 9)

Table 1: Rhythm of emergence and generative phenophases

It can be observed that time of germination in the treatment sown at the first sowing date and having no cover had no delay as compared to the germination time of the covered treatment and only 2 days earliness in beginning of harvesting time in 2013. Covering helped in earlier appearance of generative phenophases (tassels and stigma) by 4 days respectively, in case of harvesting by 3 days earliness. In the case of the plants sown at the second sowing date the emergence could be considered practically normal as common. According to our results of experiment, the male flowers (tassels) appeared in the shortest time in the transplanted treatments. The transplant growing, at this stage of development, resulted in 5 to 7 days earliness as considers the treatment P3, compared to the treatments P1 and P2 which had been direct seeded at a similar time. Compared to the control (P4), the P3 treatment transplanted at the same date, could be harvested 13 days earlier.
Results of the one of the major characteristics in connection with yield rating, unhusked and husked ear weight, are summarised in Figure 1.

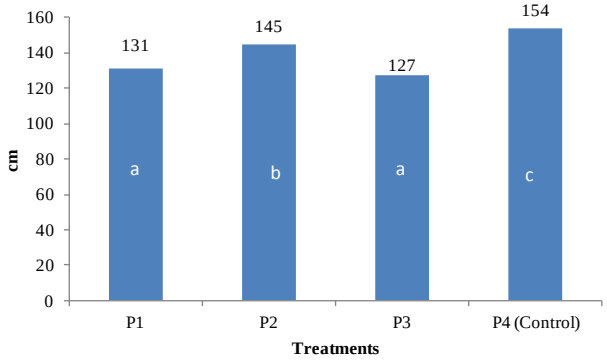


F(3;316)=25,143; F(3;316)=11,836;Sd=99%

Fig. 1. Unhusked and husked ear weight (g).

Analysing the data measured for unhusked ear yield, it is noticeable that the average weight of the ears of the transplanted, covered treatment P3 was significantly (at *p<0.01* level) lower as compared to the sowed, covered and uncovered treatments P1, P2 and P4 (control). Though there was some difference between the plants of the treatments P1 and uncovered, sowed, treatment P2 in unhusked ear weight, statistically this was not significant. Significantly highest value, supported statistically (at *p<0.01* level), of unhusked ear weight was produced by ears of control treatment (P4). In case of husked ear weight the same tendencies were observed as in case of unhusked ear weight.

The highness of plants indicator of general condition is represented by Figure 2.

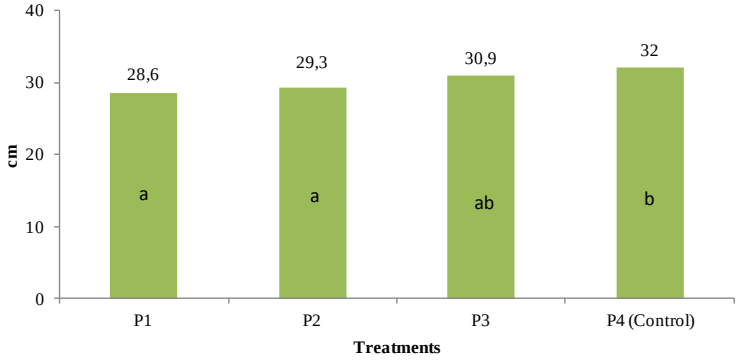


F(3;316)=61,240; Sd=99%

Fig. 2. Highness of plants (cm).

The highest value of plant highness was registered by plants of (P4 control) treatment, difference was significantly (at p<0.01 level), compared to earlier sowed (P1) covered and P2 earlier sowed (uncovered) treatments. Covering (P1) had not favourable on plant highness compared to uncovered (P2) treatment by earlier sowing time. Among covered (P1, P3) treatments plant highness no significantly difference.

Evolution of tassels length, supposed to carry more male flowers and implicitly better pollination, is summarised on Figure 3.

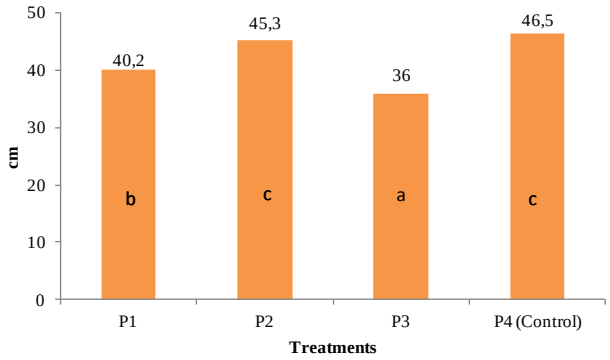


F(3;316)=16,702; Sd=99%

Fig. 3. Tassels length (cm).

In case of tassels length the time of later sowing has higher influence. Plants from later sowed uncovered, control (P4) treatments had significantly (at p<0.01 level) longer tassels compared to earlier sowed covered (P1) and uncovered (P2) treatments and longer, but not significantly, compared to transplanted covered (P3) treatment.

Highness of ear insertion is a very important morphological plant property in case of mechanical harvesting, because adapters of usual harvesting machines can cut only de sweet corn ears inserted higher than 40 cm, is illustrated on Figure 4.



F(3;316)=5,499; Sd=99%

Fig. 3. Highness of ear insertion (cm).

Obtained data confirmed significantly (at $p<0.01$ level) higher insertion of ears in case of earlier and later sowed, uncovered (P2 and P4) treatments compared to transplanted P3 treatment. Covering on earlier sowed (P1) treatment produced favourable effect on highness of ear insertion. This positive effect seems to offer possibility apply mechanical harvesting on earlier sowed sweet corn crops in open field production. Mechanical harvesting by P3 transplanted treatment can not be used.

Conclusions

Based on the results of the 2013 year experiment, the following conclusions can be made:
In case to compare the earlier sowed P1 and P2 treatments, covering resulted no effect on date of emergence, but shortened with 4 days, appaerance of generative organs (tassels and stigmina) and shortened harvesting time at least with 2 days.
The growing period was significantly shortened with transplantation of sweet corn plants compared to direct seeded. Harvest time occurred 15 days earlier in the case of transplantation and floating row cover (P3) application compared to direct sowed, uncovered, control (P4) treatment, and 8 days earlier compared to direct sowed, covered P1 treatment. At the same time the floating row cover produced 6 days shortening in the growing season between P1 (direct sowing of plants with floating row cover) and P4 (direct sowing of plants with no row cover) control treatments.
In case of direct sowed treatment (P2) the effect of covering had positive effect on unhusked, husked ear weight and highess of ear insertion.
Insertion point of ear, higher than 40 cm, offer possibility, in case of row covering in open field earlier sowing time of sweet corn crop, to use mechanical harvesting.
Covering resulted no significantly positive effect on plant highness and tassels length.

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Original scientific paper
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BIOMASS YIELD AT MAIZE UNDER DIFFERENT SOWING AND GROWING CONDITIONS

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Abstract

Maize is known as being one of the most important and most used energy crops for obtaining biomass as substrate for producing biogas. In this respect, the maize crop is important for biogas production taking into account the methane produced by one unit of dry matter, but also taking into account the high biomass yields provided by the crop. However, the high yields are possible to be obtained using the appropriate cultivation techniques according to the growing conditions. The aim of this paper is to present the biomass yield obtained at different maize hybrids under different sowing conditions (different row spacing and plant densities) and under different growing conditions (different soil and climatic conditions) from South Romania. Also, it is intended to identify the average repartition of the biomass on maize plant components under different sowing and growing conditions. Researches were performed in field experiments under rainfed conditions in the years 2013 and 2014, and in two places in South Romania. In both experimental locations and both experimental years, a number of four maize hybrids were studied under three row spacing (75 cm, 50 cm, and twin-rows of 75/45 cm) and under three plant densities (60,000, 70,000, and 80,000 plants ha^{-1}). The determinations of fresh and dry biomass were realized in the early dough - dough plant growth stage. In the specific growing conditions from South Romania, as average values for 2013 and 2014, the highest biomass yields were registered at narrow rows, especially at twin-rows of 75/45 cm, and at plant density of 80,000 plants ha^{-1}.

Key words: *maize, biomass yield, row spacing, plant density, growing conditions.*

Introduction

Maize (*Zea mays* L.) is considered as one of the most suitable crops for biomass production which can be used as substrate for producing biogas (Balodis et al., 2011; Basa et al., 2013). Most efficient utilization of maize is supply of green maize biomass directly to biogas plants for heat and power energy production (Dubrovskis et al., 2010). The maize crop is important for biogas production taking into account the methane produced by one unit of dry matter, but also the high biomass yields provided by the crop. The biomass of maize plant is depending on a sum of growth factors among which the cultivated hybrid, plant population, row spacing and soil conditions have a significant influence on the accumulation of the above-ground biomass and its repartition between plant components (Ion et al., 2014).

Selection of hybrids and FAO maturity group is of great importance in order to obtain satisfactory yield of biomass in the optimal stage of maturity even in a shorter growing season (Dubljević et al., 2013).

Row spacing and plant density are among cultivation techniques that could contribute to the production of biomass in an efficient way. Through these cultivation techniques, it is intended to reduce the intraspecific and even interspecific plant competition for acquiring the growing factors in view to be maximized the biomass production.

Maize produced in narrow rows can increase yields and result in a quicker canopy closure (Satterwhite et al., 2006). Twin-row planting systems in maize have been proposed as an

alternative spatial arrangement that should theoretically decrease plant-to-plant competition, alleviate crop crowding stress and improve yields (Robles et al., 2012).
Yields of dry biomass increase with increases in planting density (Averbeke and Marais, 1992; Yilmaz et al., 2007; Nik et al., 2011). However, this is expected to happen up to a level which could be defined as optimum plant density beyond which the dry biomass yield will start to decrease.
The aim of this paper is to present the biomass yield obtained at different maize hybrids under different sowing conditions (different row spacing and plant densities) and under different growing conditions (different soil and climatic conditions) from South Romania. Also, it is intended to identify the average repartition of the biomass on maize plant components under different sowing and growing conditions.

Materials and methods

Researches were performed in field experiments located in two places in South Romania, respectively at Fundulea (44°28’ N latitude and 26°27’ E longitude) and Moara Domneasca (44°29’ N latitude and 26°15’ E longitude). The field experiments were performed under rainfed conditions in the years 2013 and 2014.
The soil from Fundulea area is chernozem, with humus content of 2.8-3.2%, loam to clay loam texture, and pH of 6.4-6.8, while the soil from Moara Domneasca area is reddish preluvosoil, with humus content of 2.2-2.8%, clay loam texture, and pH of 6.2-6.6.
For the period April-August and at Fundulea area, the average temperature was 20.1°C in 2013 and 18.9°C in 2014, while the multiannual average temperature for this period is 18.6°C. For the same period (April-August), the sum of rainfall was 381.1 mm in 2013 and 399.0 mm in 2014, while the multiannual average rainfall is 327.9 mm.
For the period April-August and at Moara Domneasca area, the average temperature was 20.5°C in 2013 and 18.8°C in 2014, while the multiannual average temperature for this period is 18.5°C. For the same period (April-August), the sum of rainfall was 115.0 mm in 2013 and 408.0 mm in 2014, while the multiannual average rainfall is 313.2 mm.
In both experimental locations (Fundulea and Moara Domneasca) and in both experimental years (2013 and 2014), four maize hybrids were studied, respectively: Cera 450 (FAO precocity group 450), Flanker (FAO precocity group 450), PR35T36 (FAO precocity group 500), and ES Feria (FAO precocity group 550). Every hybrid in each location and in each experimental year was studied under three row spacing (75 cm, 50 cm, and twin-rows of 75/45 cm) and at three plant densities (60,000, 70,000, and 80,000 plants ha^{-1}). Each variant consisted in four lines with a length of 10 m.
In 2013, the sowing was performed on 17th of April at Fundulea location (chernozem soil) and on 26th of April at Moara Domneasca location (reddish preluvosoil). In 2014, the sowing was performed on 8th of May at Fundulea location and on 25th of April at Moara Domneasca location.
The preceding crop was sunflower in both locations and experimental years. The fertilization was performed with 106 $kg.ha^{-1}$ of nitrogen and 40 $kg.ha^{-1}$ of phosphorus. The weed control was performed by the help of herbicides, which were completed by one manual hoeing.
In each location, in each experimental year, and from each variant the maize plants from one square meter were cut at soil level and were weighed immediately in view to be determined the fresh biomass yield, respectively the yield of above-ground biomass. One average maize plant for each variant was taken into the laboratory and dried in the oven at 80°C for 24 hours in view to be determined the dry biomass yield.
Determinations were performed in the early dough - dough plant growth stage, respectively in the growth stage when the maize biomass is of importance to be used as substrate for

producing biogas. The data are presented and analyzed as average values for the four studied maize hybrids.

Results and discussion

Biomass yield at maize under different row spacing conditions. On chernozem soil, the highest biomass yields were registered at narrow rows. Thus, in average for 2013 and 2014 it was obtained 72.82 tons.ha^{-1} of fresh biomass at 50 cm between rows (Figure 1.a) and 20.71 tons.ha^{-1} of dry biomass at twin-rows of 75/45 cm (Figure 1.b). The same tendencies were registered also on reddish preluvosoil, where the highest yields were 55.59 tons.ha^{-1} of fresh biomass at 50 cm between rows (Figure 1.a) and 20.15 tons.ha^{-1} of dry biomass at twin-rows of 75/45 cm (Figure 1.b).

The biomass yields were higher on chernozem soil conditions respectively at Fundulea location, which was characterised by more rainfalls and smaller values of temperatures compared to the conditions registered on reddish preluvosoil at Moara Domneasca location. In average for 2013 and 2014 and in average for the three variants of row spacing, the fresh biomass yield was of 69.84 tons.ha^{-1} on chernozem soil and of 53.36 tons.ha^{-1} on reddish preluvosoil, while the dry biomass yield was of 19.89 tons.ha^{-1} on chernozem soil and of 19.28 tons.ha^{-1} on reddish preluvosoil (Figure 1). The smallest variations between the two experimental years regarding the yield of dry biomass at different row spacing were registered on reddish preluvosoil.

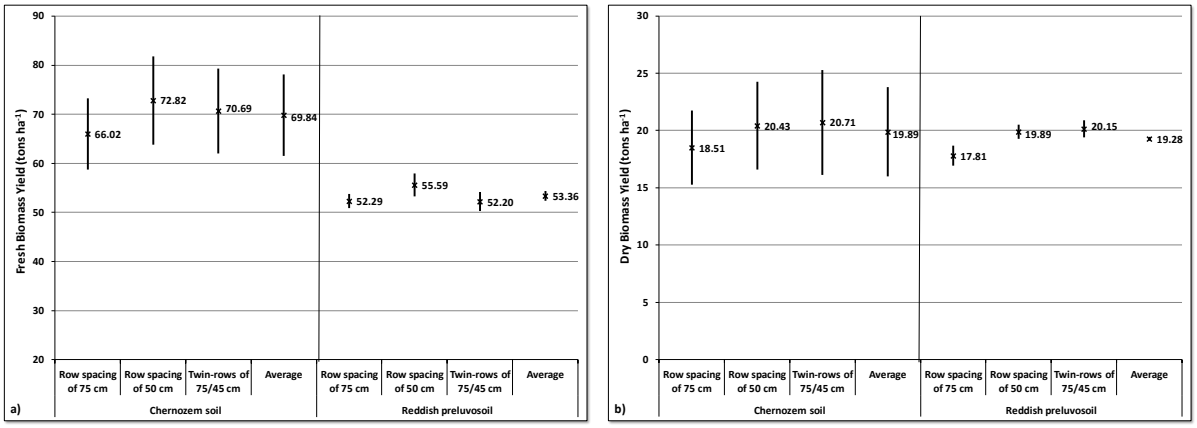

Figure 1. Fresh (a) and dry (b) biomass yield at maize under different row spacing conditions, on different types of soils from South Romania, as average values and limits of variations in 2013 and 2014 climatic conditions

Biomass yield at maize under different plant density conditions. Increasing of plant density from 60,000 to 70,000 and further to 80,000 plants ha^{-1} determined the increasing of fresh and dry biomass yield (Figure 2). In average for 2013 and 2014, the highest yields of dry biomass were registered at plant density of 80,000 plants ha^{-1} both on chernozem soil (20.32 tons.ha^{-1}) and reddish preluvosoil (20.18 tons.ha^{-1}), while the highest yield of fresh biomass was registered at 80,000 plants ha^{-1} on chernozem soil (70.35 tons.ha^{-1}) and at 70,000 plants ha^{-1} on reddish preluvosoil (55.53 tons.ha^{-1}).

In average for the two experimental years (2013 and 2014) and for the three variants of plant density (60,000, 70,000 and 80,000 plants ha^{-1}), the fresh and dry biomass yields were higher on chernozem soil then on reddish preluvosoil. As in the case of fresh and dry biomass yields registered at different row spacing, the smallest variations between the two experimental years were registered on reddish preluvosoil (Figure 2).

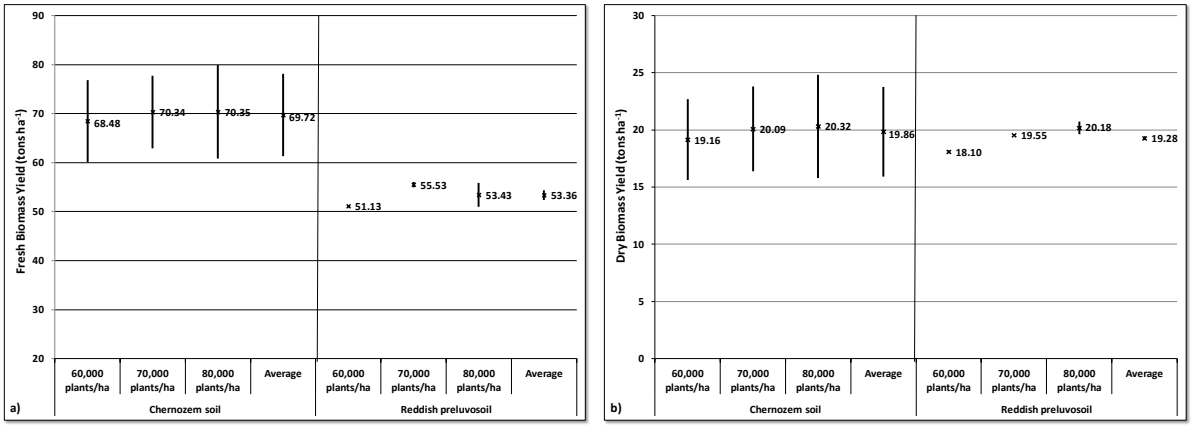

Figure 2. Fresh (a) and dry (b) biomass yield at maize under different plant density conditions, on different types of soils from South Romania, as average values and limits of variations in 2013 and 2014 climatic conditions

Share of dry biomass on plants components at different row spacing. Regarding the share of dry biomass on stalks (including leaf sheaths and tassels), leaves (leaf blades), ears and husks in the early dough - dough plant growth stage, the highest ration were registered by the ears both on chernozem soil and reddish preluvosoil and both at different row spacing and plant densities (Figures 3 and 4).

On chernozem soil, the narrow rows increased the share of dry biomass on ears and leaves and decreased the share of dry biomass on stalks and husks (Figure 3.a). On reddish preluvosoil, compared to the shares registered at 75 cm between rows, the row spacing of 50 cm increased the share of dry biomass on stalks, leaves and husks, while the twin-rows of 75/45 cm increased the share of dry biomass on ears and husks (Figure 3.b).

In average for the two experimental years (2013 and 2014) and for the three variants of row spacing (75 cm, 50 cm and twin-rows of 75/45 cm), on chernozem soil the shares of dry biomass on plant components were the following: 44.8% for ears, 32.7% for the stalks, 15.0% for the leaves, and 7.6% for the husks (Figure 3.a). On reddish preluvosoil, the average shares of dry biomass on plant components were the following: 48.7% for ears, 29.5% for the stalks, 14.8% for the leaves, and 7.0% for the husks (Figure 3.b). So, on reddish preluvosoil in average it increased the share of dry biomass on ears and decreased the share of dry biomass on all other plant components compared to the values registered on chernozem soil.

Share of dry biomass on plants components at different plant densities. On chernozem soil, the increasing of plant density from 60,000 to 70,000 and further to 80,000 plants ha^{-1} increased the share of dry biomass on stalks and leaves and decreased the share of dry biomass of ears and husks (Figure 4.a).

On reddish preluvosoil, the increasing of plant density increased the share of dry biomass on ears and leaves and decreased the share of dry biomass of stalks and husks (Figure 4.b).

In average for the two experimental years (2013 and 2014) and for the three variants of plants densities (60,000, 70,000, and 80,000 plants ha^{-1}), on chernozem soil the shares of dry biomass on plant components were the following: 44.9% for ears, 32.6% for the stalks, 15.0% for the leaves, and 7.5% for the husks (Figure 3.a). On reddish preluvosoil, the average shares of dry biomass on plant components were the following: 48.8% for ears, 29.5% for the stalks, 14.8% for the leaves, and 7.0% for the husks (Figure 3.b).

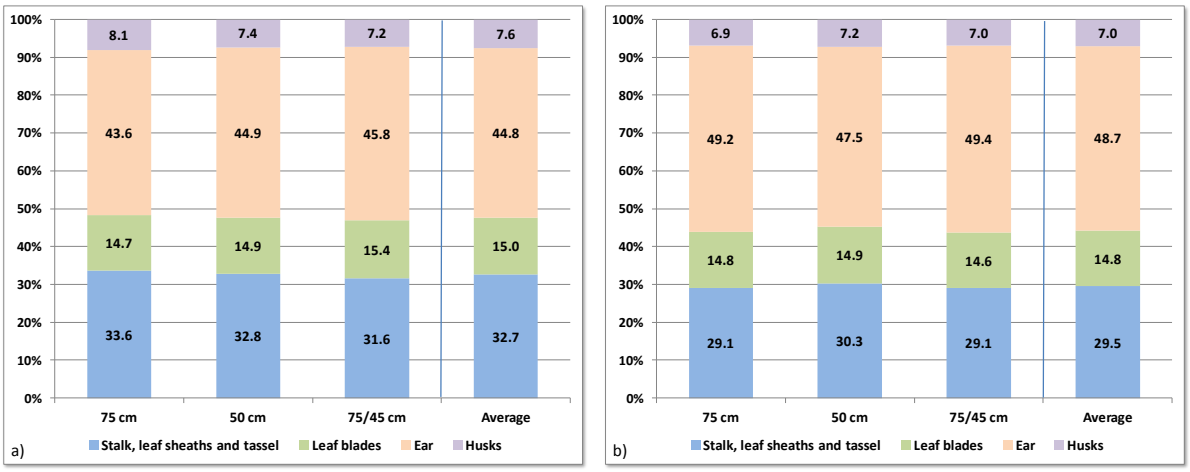

Figure 3. Percentage of dry biomass on maize plant components under different row spacing, on different types of soils (a- chernozem soil; b- reddish preluvosoil) from South Romania, as average values in 2013 and 2014 climatic conditions

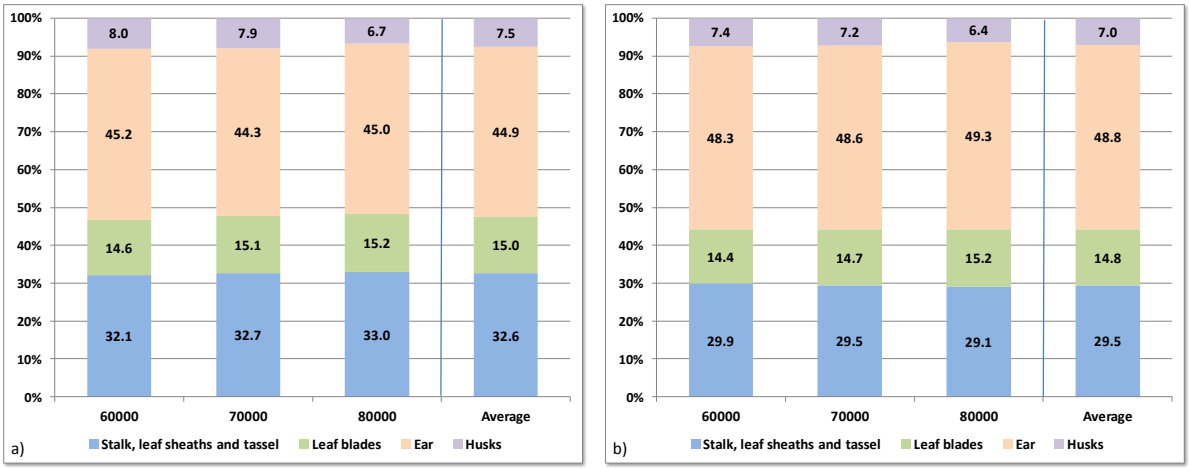

Figure 4. Percentage of dry biomass on maize plant components under different plant densities, on different types of soils (a- chernozem soil; b- reddish preluvosoil) from South Romania, as average values in 2013 and 2014 climatic conditions

Conclusion

In the specific growing conditions from South Romania, as average values for 2013 and 2014, the highest biomass yields were registered at narrow rows, especially at twin-rows of 75/45 cm, and at plant density of 80,000 plants ha^{-1}.

The biomass yields were higher on chernozem soil conditions, which were associated with more rainfalls and smaller values of temperatures compared to the conditions registered on reddish preluvosoil.

Among the plant components, the highest ration of dry biomass were registered by the ears both on chernozem soil and reddish preluvosoil conditions, as well as under different row spacing and plant densities.

On chernozem soil, the narrow rows increased the share of dry biomass on ears and leaves, while on reddish preluvosoil the row spacing of 50 cm increased the share of dry biomass on stalks, leaves and husks, and the twin-rows of 75/45 cm increased the share of dry biomass on ears and husks.

On chernozem soil, the increasing of plant density increased the share of dry biomass on stalks and leaves. On reddish preluvosoil, the increasing of plant density increased the share of dry biomass on ears and leaves.
Compared to the average values registered on chernozem soil, on reddish preluvosoil the share of dry biomass on ears increased while the share of dry biomass on all other plant components decreased.

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Original scientific paper
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ANALYSIS OF THE YIELD COMPONENTS AT MAIZE UNDER THE SPECIFIC CONDITIONS FROM SOUTH ROMANIAN

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Abstract

The total grain production of maize reaching 11.34 million tons in 2013 placed Romania on the second position in the EU. In this context, selecting of the proper hybrid for each agricultural region is an essential condition for farmer to achieve their production goals.
The purpose of the paper was to present the results regarding the yield components at different maize hybrids under the specific conditions from the South Romanian Plain. In this respect, there have been studied an assortment of eleven maize hybrids under the conditions of the cambic chernozem from Baneasa area (44o03’ N latitude and 26o05’ E longitude), Giurgiu County, under the favourable climatic conditions of 2014. The hybrids tested in the comparative crop were the following: PR37Y12, Rapsodia, Olt, PR35F38, P0216, P0412, Generos, Fundulea 376, Florencia, P0105, Kitty. At full maturity stage, it was determined the total biomass, the yield components, the grain yield, and the harvest index. The maize hybrids react considerably function of their precocity, plant density, fertilization strategy, weed control and rainfall distribution during vegetation phases.
Key words: *maize hybrids, growing conditions, yield, yield components.*

Introduction

Assortment of maize hybrids have a high yield potential, but the crop technology and the climatic conditions have a considerable influence on the yield components and the average yield of hybrids. The maize hybrids have a different behaviour according to cultivation area, soil and climatic conditions, rainfall distribution during phases of vegetation, used fertilization dosage, weed control and the plant density according to the field conditions (Dumbravă, 2004). The yield components are influenced by the hybrids selected for cultivation in an area and the fertilizer doses used (Adeyemi, 2011). The effect on sowing on plant density expressed that delay in sowing reduces the number of kernels in cob and performance components of maize (Cantanero et al., 2000). Early and intermediate sowing tends to best utilize solar radiation for grain production (Koca and Canavar, 2014). This could be due to a better growing and development of the crop which is a consequence of a good photosynthesis products accumulation in leaves and their transfer to economic components of the of plants like cobs and grains (Otegui et al., 1995). The plant density correctly established function of hybrid precocity, fertilization doses, weed control and water reserve in soil has a considerable influence on the competition among the plant populations which use the natural resources and determines the yield components for each hybrid (Sharifi et al., 2009).
The aim of this paper was to present the results related to the yield components, total biomass and grain productions for maize hybrids under the conditions of performance crop technology and under pedoclimatic conditions from Romanian plain.

Materials and methods

The comparative hybrid crop was located in an experimental field on cambic chernozem in Baneasa Giurgiu County under the specific climatic condition of 2014. The annual amount of rainfall was 450 mm in the period April-September, with a good distribution to second decade of July, and the average annual temperature was 11.8 C.

Studied maize hybrids were the following: PR37Y12 (FAO 380), Rapsodia (FAO 400), Olt (FAO 400), PR35F38 (FAO 450), P0216 (FAO 450), P0412 (FAO 480), Generos (FAO 500), Fundulea 376 (FAO 500), Florencia (FAO 490), P0105 (FAO 400), Kitty (FAO 490).

Each hybrid had 4 rows with a length of 10 m, 4 replications. Seeding data was 7 April, and plant density was of 55,000 harvestable plants for hectare. The crop technology was the following: the preceding crop was wheat, the fertilization was performed with 100 kg/ha of complex 20:20:0 and 150 kg/ha of ammonium nitrate at the 7 leaf stage; the weed control was performed postemergent with the following herbicides: Dicopur Top 464 SL (344 g/l acid 2.4 D from SDMA + 120 g/l dicamba) at a rate of 1 l/ha, and Novapower 40 SC (nicosulfuron 40 g/l) at a rate of 1.5 l/ha, which were applied at 5 leaves stage of maize plants.

At harvesting determination and analysis were performed regarding: total biomass maize production, weight of grains per ha, grain ratio on cob, number of grains per ear, grain yield, grain humidity, the hectoliter mass, mass of 1000 grains and standard yield at 15% humidity.

Experimental data were processed using the analyses of variance.

Results and discussion

Above-ground biomass. The plants were cut at soil surface at harvesting and the above-ground biomass was calculated after weighing.

The highest total biomass yield was recorded for the hybrids P0216 (24.8 t/ha), F376 (22.2 t/ha) and Florencia (21.3 t/ha). The lowest biomass was recorded for hybrids Rapsodia (16.1 t/ha) and PR37Y12 (16.4 t/ha). In the comparative crop, 6 hybrids achieved total biomass yield higher than the average for the experiment (Figure 1).

The total biomass yield was boosted by the favorable pedoclimatic and technologic conditions but the high temperature and rainfall shortage in July and August had influences on the yield potential for the tested hybrids.

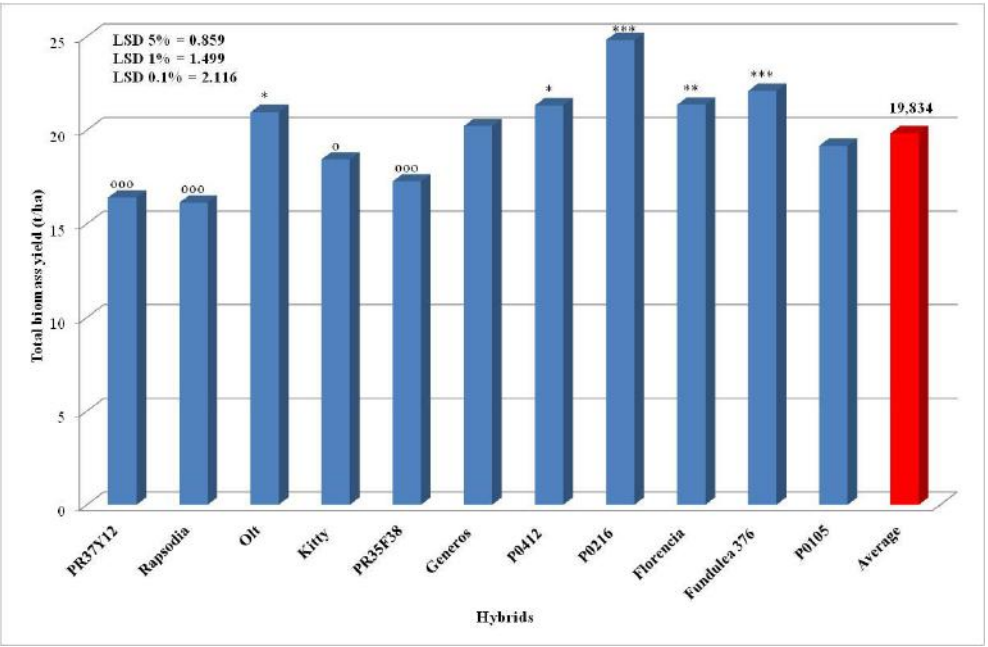

Figure 1. Total biomass yield

The maize ear yield. The average ear yield was 10.4 t/ha with significant variations among hybrids. The highest ear yield was registered at hybrids P0216 (13.8 t/ha), Florencia (13.7 t/ha) and F376 (13.6 t/ha), with grain humidity between 17.7% and 18.5%.
Lower ear yield were recorded for the hybrids PR37Y12 (9.4 t/ha), Rapsodia (9.5 t/ha) and PR35F38 (10.7 t/ha) (Figure 2).

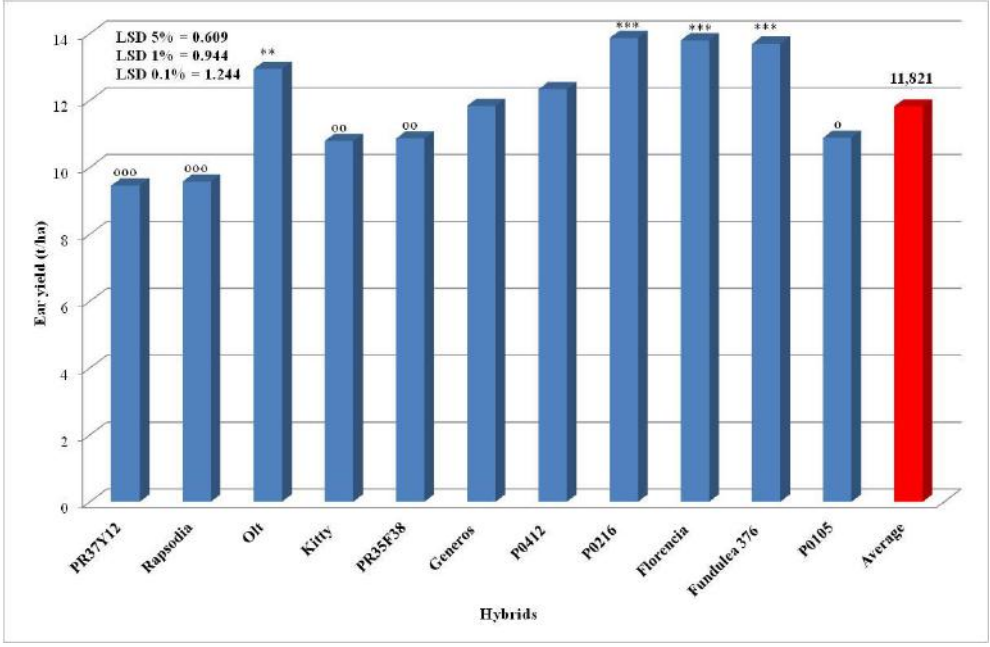

Figure 2. The maize ear yield

The average weight of maize ear. The average weight of maize ear is correlated with number of grains per ear, grain mass per ear, grain humidity, grain yield, and grain ratio on ear.
For the eleven studied hybrids the average weight of ear was 209.33g. There were remarkable the hybrids P0216 with 254.11 g and a grain ratio on ear of 83.5%, Florencia with 247.11 g and grain ratio on ear of 80.2%, and P0412 whit 225.5g and grain ratio on ear of 82.3%. Lower value of the weight of maize ear was 170.80g for PR37Y12 with a grain ratio on ear 81.3% and 171.24g for Rapsodia and grain ratio on ear 80.5% (Figure 3).

Number of grains per ear. Number of grains per ear is correlated with mass ear, grain ratio on ear, grain humidity and mass of 1000 grains. For the eleven studied hybrids the average number of grains on ear was 562.45 grains.
The number of grain per ear was influenced by hybrid. We noticed a variation from 592 grains per ear for Rapsodia hybrid to 630 grains per ear for PR35F38 and significantly value exceed the average the hybrids PR35F38, Kitty, and F378.

Grain weight per ear. The grain mass per maize ear was influenced by the ear mass, grain size on ear, grain ratio on ear and grain humidity.
We noticed a variation of the grain mass per ear from 137.89 g at Rapsodia hybrid to 212.37 g at PO216 hybrid. The big significantly value exceed the hybrids PO216 with 212.37 g, Florencia with 198.3 g and PO412 with 185.86 g. Lower values of the grain mass per ear were recorded for the hybrids Rapsodia, PR35F38, and PR37Y12. The harvest plant density influenced directly the grain yield per ear.

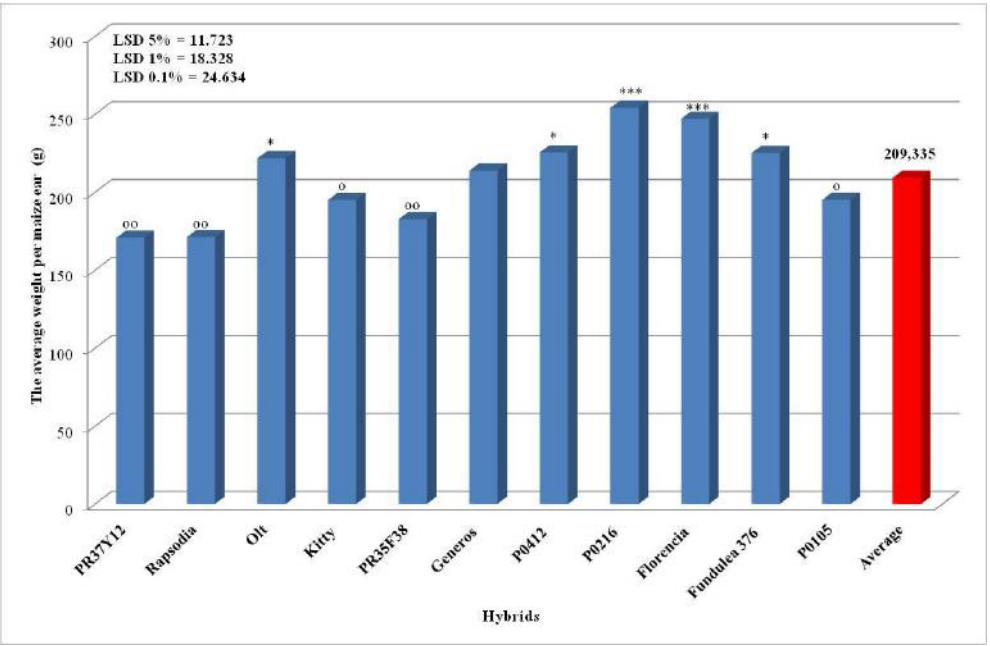

Figure 3. The average weight of maize ear

Grain ratio on ear. The grain ratio on ear is influenced by the grains mass per ear, grains humidity and maize ear mass. The grain ratio on ear is not significantly different for the eleven tested hybrids in experimental field. Based on this indicator, we point out the hybrids P0216 with grain ratio on ear 83.5%, P0412 (82.3%), and P0105 (81.9%). For the Romanian hybrids, the grain ratio on ear had low values, respectively Olt (77.1%) and F 376 (79.5%), and that is because grains humidity was of 18.5 % for these hybrids.

Mass of 1000 grains. The mass of 1000 grains is significantly different on hybrids and it is determinate by grain mass on ear, number of grains on ear, grains humidity and climatic conditions in the grain formation period.
The highest mass of 1000 grains was recorder for the hybrids P0216 (412.5g), Florencia (395.2 g), and F376 (375.1g).
The lowest mass of 1000 grains was recorded in the case of hybrids PR35F38 (260.6g) and PR37Y12 (271.9g).

Grain humidity. The grain humidity is correlated to the hybrid precocity and the climatic conditions in the cultivated area. For the eleven tested hybrids in the experimental field, grains humidity is variable from 16.5% for PR37Y12 hybrid to 18.5% for Olt and F376 hybrids. The foreign maize hybrids for Romania had the ability to lose grain humidity fast at plant maturity.

Standard yield of grains at the humidity 15%. The average standard yield for the hybrids tested in Romanian plain conditions in 2014 year was 9.3 t/ha.
The highest standard yield were recorded for the maize hybrids P0216 (11.1 t/ha), Florencia (10.9 t/ha), and F376 (10.5 t/ha), which significantly exceed the average yield per experiment. Lower yields, significantly distinct from the average yield were recorded for the hybrids Rapsodia (7.5 t/ha) and PR37Y12 (7.5 t/ha).
The higher temperatures and rainfall deficit in July and August affected the full potential yield of the tested hybrids.

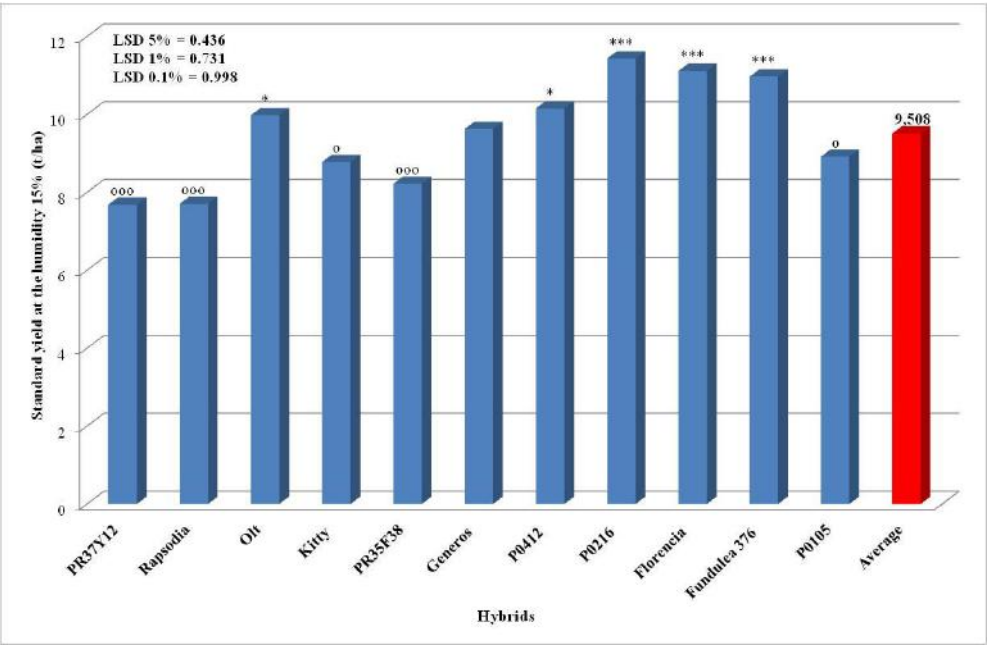

Figure 4. Standard yield of grains at the humidity 15%

Conclusion

The maize hybrids react considerably function of their precocity, plant density, fertilization strategy, weed control and rainfall distribution during vegetation phases.

The aggressive temperatures in the context of rainfall deficit in July and August have a considerable influence on the yield components regardless of the tested hybrids in the climatic conditions specific of the 2014 year in South Romanian Plain.

Farmers growing maize must consider crop technology management for a maximum use of pedoclimatic resources and for the decrease in effect of limitative factors on yield components.

Due to the large biomass production at maize, it is of interest to manage the crop residues after harvesting and by the new technology that can be used to produce pellets or briquettes for heating units with considerable economic effects.

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INFLUENCE OF SPRING AGROTECHNICAL CARE TECHNIQUES ON FESCUE LAWN GRASS STAND QUALITY

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Abstract

Creation of landscape composition on the plot, the park or near administrative buildings only with trees, shrubs and flower beds will lead to the creation of an unfinished composition. Perennial ornamental grasses red fescue (*Festuca rubra*) and fescue (*Festuca pratensis*) in the climatic characteristics of Preduralie are excellent herbs for creating long-term lawns, both in pure form or in mixes. Sod formed from both fescues has great connectivity, density and elasticity, with dark green leaves that do not lose their coloring even in drought periods.

Application in spring harrowing or rolling grass herbage can improve quality, reduce the amount of lawn weeds in grass stands. Application of agrotechnical care methods (harrowing, rolling) during regrowth of lawns in spring allows improving the living conditions of the plants and form a dense grass stand of good quality.

It is also worth mentioning that having reached full development in the third year of life, the grasses respond better to rolling and harrowing-rolling combination during regrowth period.

The density of grass stand increases compared to the variants without treatment and harrowing with tooth harrows on average from 220 to 2227 shoots/m^2 over the years of research.

Sowing grass mixture 50% red fescue and 50% fescue, using rolling or harrowing combined with rolling in spring enables the lawns of good and excellent quality.

Keywords: *lawns, red fescue, meadow fescue, harrowing, rolling.*

Introduction

Landscape as an environment in which human operates permanently is of great importance. Lawn in landscape design is not only a decoration, it is a place for children to play, to have rest and picnics. Creation of landscape composition on the plot, the park or near administrative buildings only with trees, shrubs and flowerbeds will lead to the creation of an unfinished composition. Use of grasses that are adapted to climatic conditions of a region, resistant to temperature fluctuation allows lawns of satisfactory and good quality since the first year of plant life. Perennial ornamental grasses red fescue (*Festuca rubra*) cv. Sverdlovskaya and meadow fescue (*Festuca pratensis*) cv. Sverdlovskaya-37 in the climatic characteristics of Preduralie are excellent plants for creating long-term lawns, both in pure form or in mixes. Sod formed from the fescue has great connectivity, density and elasticity, with dark green leaves that do not lose their coloring even in drought periods (Lazarev, 2011). Application of harrowing and rolling in the spring period allows improving the quality of lawns, reducing the quantity of weed herbs in grass stand.

Since for the region of Preduralie there are no recommended plants and no developed agrotechniques for creating long-term lawns of good quality, the studies on the fescues are topical for further recommendations to landscape design companies.

Materials and methods

The investigations of agrotechniques for lawns are carried out in the experimental field of the Perm State Agricultural Academy, Perm, Russia, in particular, mono-sowings of red fescue

and mixture of red fescue and meadow fescue (50/50) in spring, during regrowth period (since the second year of life) agrotechnical methods of care: harrowing without harrows, rolling with star-wheel rollers and combination of these techniques.

Table 1

Experiment scheme «Influence of care technique on forming of sod from perennial grasses and lawn quality»

Without treatment	Harrowing	Rolling	Harrowing+ rolling
red fescue 100%			
red fescue 50% + fescue 50%			

The most common ornamental grasses in the region: meadow fescue (Sverdlovskaya-37) and red fescue (Sverdlovskskaya), perennial ornamental grasses of winter type of development were taken for the study. Meadow fescue and red fescue possess good winter hardiness and cold resistance (Kobozev et al, 2002). Meadows dominated by numerous radical vegetative shoots, which respond well to regular cuts, forming dense durable plant (Subbotina, 2006; Tiuldiukov, 2002)

The area of plot is 24 m^2, planted four times, completely randomized placing. Soil of the plot is sod-podsolic (typical for Preduralie), organic matter content 2.3 – 2.4%; pH=5.0; P2O5=120-185 mg/100 g; K2O=145-160 mg/100 g. Seeding rate in its pure rate with 100% germination: red fescue 13.3 g/m^2; meadow fescue 18 g/m^2. Sowing was carried out in the third decade of May manually. Shearing was carried out with a lawn mower at intervals of 14 days. The quantity of plants and shoots was calculated twice a vegetation period by monolith methods (quantitative calculating of plants) (Kobozev et al., 2002).

Results and discussion

In the first year of life, lawn shoots were not simultaneous, and by the end of the vegetation period the number of shoots on the experience, on average, was approximately 4000 per square meter with projective covering 60-65% (Table 2). For the second year of life, quality of lawns herbage has improved greatly and on studied options on average amounted to 5008-6659 shoots/m^2 with projective covering 75-84%. Red fescue seeding in pure stand from the second year of life helps form the lawns of satisfactory quality with 6350-6842 shoots/m^2 and projective covering 83-86%. The combination of red fescue and meadow fescue with 50/50 provides 4775-5475 shoots/m^2 with projective covering 70-81%.

Starting with the third year of life, the quality of grass stands was much better in the versions with sowing red fescue (*Festuca rubra*) + fescue (*Festuca pratensis*), the number of shoots per square meter in the options above at 586-1407 pieces/m^2 with roughly the same projective covering 72-76%. By the fourth year of life the studied lawns projective coverage amounted to 81-83% and 7001-7758 shoots per square meter in red fescue in pure stand and red fescue and fescue, respectively.

Lawn coverage made of fescue mixture forms on the 132-1400 shoots/m^2 more both when harrowing is applied during spring or rolling of herbage growing and without processing.

Table2

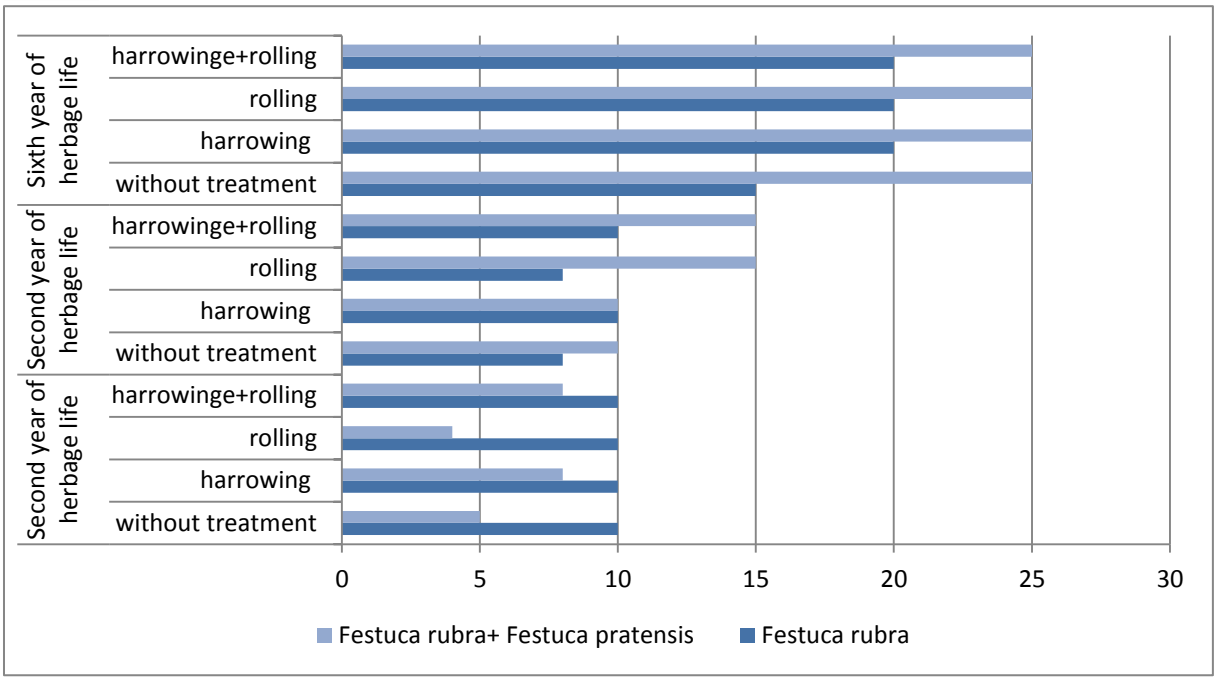
Influence of spring agrotechnical care techniques on fescue lawn quality

Year of herbage life	Care techniques in the regrowth period	*Festuca rubra*		*Festuca rubra+ Festuca pratensis*	
		quantity of shoots, pieces/m^2	projective covering, %	quantity of shoots, pieces/m^2	projective covering, %
Second	without treatment	6625	83	4775	81
	harrowing	6350	86	5225	70
	rolling	6842	82	4558	75
	harrowinge+rolling	6817	85	5475	75
Average		***6659***	***84***	***5008***	***75***
Fourth	without treatment	6891	80	7023	82
	harrowing	7112	82	7554	82
	rolling	6998	80	8398	84
	harrowinge+rolling	7002	83	8055	84
Average		***7001***	***81***	***7758***	***83***
Sixth	without treatment	8698	97	10157	97
	harrowing	9254	98	10349	99
	rolling	10598	95	12576	100
	harrowinge+rolling	9427	99	10886	100
Average		***9494***	***97***	***10992***	***99***

In following years of research, thanks to systematic grass density cuts is constantly increasing and the quality of the grass coating remains good with projective covering up to 100% and the number of shoots to 12576 pieces/m^2. In the sixth year of lawns life, red fescue in pure form and mixture of red fescue and fescue are highlighted in the treatments with the use of rolling in the spring regrowth period. Red fescue formed to the sixth year of life 10598 shoots/m^2 and projective coverage 95%, mixed with fescue, number of shoots formed was much higher - 12576 shoots/m^2 and 100% of the projective cover. Also it may be noted that conducting agrotechnical measures affects the quality of the lawn in different years of herbage. Methods of care are applied since the second year of lawns life to make grasses fairly well root, to avoid damaging them. Red fescue seeding in its pure form in all the years of research responded positively to the application of all treatments: harrowing, rolling, and harrowing-rolling combination. Red fescue and fescue mixture responds positively to spring harrowing, starting since the third or fourth year of life.

In general, the lawns of satisfactory quality were obtained from the second year of life both red fescue planted in its pure form, and fescue and red fescue planted in a mixture of 50/50.

Upon further examination of the herbage it was observed that to the sixth year of life the use of red fescue in pure form allows creating good quality lawns; mixture of red fescue and fescue50/50 improves the quality of lawn to excellent (Picture 1).



Picture 1. Influence of spring agrotechnical care methods on fescue lawn grass stand quality

Conclusion

According to the research results on possibility to create longstanding lawns of red fescue in pure form and in mixture using agrotechnical methods in spring period following conclusions can be drawn:

The optimum is to use a mixture of red fescue (*Festuca rubra*) variety Sverdlovskaya and fescue (*Festuca pratensis*) variety Sverdlovskaya -37.

Application of agrotechnical methods of care during regrowth of grass herbage allows increasing the density of red fescue grass by 107-1900 shoots/m^2, in mixture of fescue and red fescue by 192-2419 shoots/m^2thanks to of more intensive root stock in the fourth to sixth year of the studies.

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AGROECOLOGICAL IMPORTANCE OF FIELD CROP ROTATIONS IN PERM REGION SOIL AND CLIMATIC CONDITIONS

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Abstract

Typical grain-fallow crop rotations and ones, highly filled with legumes, were studied in stationary field experiment during 1998-2014 years on sod-podzolic soil (*Umbric Albeluvisols Abruptic*) on the backgrounds: without fertilizers, organic, mineral and organic-mineral fertilization systems. Crop rotations with green-manured fallow, annual legumes and perennial legume grasses proved their importance for promotion of high total yields and soil fertility raising. The same effect of typical grain-fallow crop rotations was achieved only with extremely high rates of FYM and mineral fertilizers.

Positive effect of joint application of manure and mineral fertilizers was noted in the experiment. Stabilization of soil fertility in typical crop rotation was achieved in treatment with manure application 60 t ha^{-1} in combination with NPK, in rotations with legumes – in the treatment with 40 t ha^{-1} and $N_{60}P_{60}K_{60}$. Organic-mineral fertilizer system provides not only the formation of positive balance of humus, but also its quality improvement.

Crop rotations with green-manured fallow and legumes provide stable high crops productivity. Application of 40 t ha^{-1} FYM and $N_{60}P_{60}K_{60}$. permitted to raise average crops productivity up to 3140 feed units (FU) t ha^{-1}, grain yields – 3,0 t ha^{-1}`, the energy ratio – 2,59. The supplements compared with typical rotations varied from 480 to 1190 FU per hectare.

Keywords: *crop rotation, green-manured fallow, perennial legume grasses, farmyard manure (FYM), mineral fertilizers*

Introduction

Sod-podzolic soils are most common in Perm region and are characterized by low level of natural fertility. They are featured by high soil acidity, low organic matter content, unfavourable physical and chemical properties. Using crop rotations with green-manured fallow, annual legumes and perennial legume grasses is the most available way for increasing fertility of these soils. It provides activation of fertility biological factors, reduces the use of organic and mineral fertilizers, increases the biocenosis productivity.

Crop rotations are important part of sustainable agriculture, which allows to optimize the functioning of agro- biocenosis based on rational use of natural, biological and anthropogenic factors (Kozlov and Abashev 2004, Thompson and Whitney, 2000). Scientifically founded crop rotations, adapted to the specific landscape, are most available means of soil fertility recovery (Lykov, 1982). Using of green-manured fallows, annual legumes and legume perennial grasses in crop rotations allows to activate fertility biological factors, reduce the use of mineral fertilizers and pesticides, while maintaining the productivity of arable land at high level, according to Loshakov (2012) and others.

Crop rotation eliminates soil degradation, which is associated with soil depletion of macro- and micronutrients, enzyme activity decreasing, accumulation of nematodes and other phytopathogenic microorganisms in soil (Naumkin et al., 2010; Loshakov, 2012; Bartels, 2006; Leithold and Volk, 2007).

Modern situation in Russian agriculture and, particularly, in Ural region, demands scientifically founded crop rotations, adapted to the specific soil and climatic conditions. Therefore, the purpose of given research is the assessment of different field crop rotations aimed at raising of arable crops productivity, soil fertility preservation and ecological safety.

Materials and methods

The experimental work was fulfilled in 1995-2014 in long-term stationary field experiment on the experimental farm of Perm Scientific Research Institute. The experimental plots located on sod-podsolic heavy loam soil with humus content 2.13-2.21 %, pH – 5.4, P_2O_5 – 100 mg kg^{-1}, K_2O – 150 mg kg^{-1}. (1969, before experiment foundation).

Experimental scheme: factor A – crop rotations: 1. Typical eight-course field rotation with bare fallow, rotation scheme: bare fallow, winter rye, spring wheat as shelter crop for clover (*Trifolium pretense* L.) and timothy (*Phleum pretense* L.) mixture, first year clover-timothy mixture, second year clover-timothy mixture, spring wheat, silage crops (sunflower with spring rape), spring wheat; 2. Eight-course field rotation with green-manured fallow, rotation scheme: green-manured fallow (mixture of spring vetch and oat), winter rye, spring wheat as shelter crop for meadow clover, first year clover, spring barley, spring wheat as shelter crop for meadow clover, first year clover, spring wheat. Factor B – fertilizers application: 1. No fertilizers (control); 2. Mineral fertilizers – NPK 30, 60, 90 kg ha^{-1}; 3. Farm-yard manure (FYM) 20, 40, 60 t ha^{-1}; 4. FYM 20, 40, 60 t ha^{-1} + NPK 30, 60, 90 kg ha^{-1}. Treatment placing is randomized, each treatment has four replications.

FYM were applied in bare and green manuring fallow, mineral fertilizers –for grain and fodder crops. Their afteraction was studied on clover and mixture of clover and timothy.

Soil and plant chemical analyses were fulfilled in analytical laboratory of Perm Agricultural Research Institute according national standards: humus content – GOST 26213-84 (Tyurin method); pH_{KCl} – GOST 26483-85 (potentiometrically); hydrolytic acidity - GOST 2 OST 26207-91 (Kappen method); total exchange bases – GOST 27821-88 (Kappen-Gilkovits method); content of mobile phosphorus and exchange potassium – GOST 26207-91 (Kirsanov method). Data processing included analysis of variance and correlation coefficients determination, using the program SPSS (v.18).

Results and discussion

Chemical parameters change of soil used in agriculture is significant aspect of their ecological assessment.

The agricultural use of soil without fertilizers application for eight years led to deterioration of soil absorbing complex parameters in plowing horizon in typical and green manuring crop rotations. Tendency of soil acidification in both rotations was noted, caused by pH_{KCl} decrease at 0.4-0.2, total exchange bases – at 28-30 mmol kg^{-1}

Concealed degradation processes were accompanied by increasing not only actual, but also potential acidity. Hydrolytic acidity increased by 2-3 mmol kg^{-1}. These processes were less expressed in green manuring crop rotation.

Degradation processes revealed also in decrease of humus content, average losses were 0.25 % in typical crop rotation and 0.02 % in green manuring crop rotation (table. 1). Less humus losses in green manuring rotation caused by green manure plowdown in fallow field.

Table 1. Effect of fertilizers and crop rotations on soil chemical properties.

Treatment	Typical crop rotation				Green manuring crop rotation			
	humus, %	pH_{KCl}	P_2O_5, mg kg^{-1}	K_2O, mg kg^{-1}	humus, %	pH_{KCl}	P_2O_5, mg kg^{-1}	K_2O, mg kg^{-1}
Original soil	2.13	5.5	107	149	2.21	5.4	104	152
No fertilizers	1.88	5.1	88	123	2.19	5.2	91	134
$N_{30}P_{30}K_{30}$	1.91	5.3	88	128	2.17	5.0	98	130
$N_{60}P_{60}K_{60}$	1.81	5.1	95	146	2.17	5.1	104	156
$N_{90}P_{90}K_{90}$	1.87	5.0	120	141	2.26	5.2	113	161
FYM 20 t ha^{-1}	1.86	5.2	83	130	2.13	5.2	89	125
FYM 20 t ha^{-1} + $N_{30}P_{30}K_{30}$	1.90	5.6	88	126	2.22	5.1	94	136
FYM 20 t ha^{-1} + $N_{60}P_{60}K_{60}$	1.91	5.1	110	137	2.17	5.0	109	157
FYM 20 t ha^{-1} + $N_{90}P_{90}K_{90}$	1.90	5.2	113	149	2.20	5.0	115	163
FYM 40 t ha^{-1}	2.00	5.4	102	130	2.22	5.2	95	145
FYM 40 t ha^{-1} + $N_{30}P_{30}K_{30}$	2.01	5.4	96	138	2.27	5.0	95	145
FYM 40 t ha^{-1} + $N_{60}P_{60}K_{60}$	1.96	5.3	109	147	2.26	4.9	121	155
FYM 40 t ha^{-1} + $N_{90}P_{90}K_{90}$	1.96	5.1	136	163	2.32	5.0	125	163
FYM 60 t ha^{-1}	2.24	5.4	94	142	2.38	5.1	109	146
FYM 60 t ha^{-1} + $N_{30}P_{30}K_{30}$	2.24	5.4	104	151	2.27	5.1	108	146
FYM 60 t ha^{-1} + $N_{60}P_{60}K_{60}$	2.20	5.3	115	161	2.34	5.0	128	168
FYM 60 t ha^{-1} + $N_{90}P_{90}K_{90}$	2.24	5.3	136	166	2.33	4.9	142	170
Average	1.99	5.3	105	142	2.24	5.1	108	150
LSD_{05}	0.11	0.4	12	18	0.10	0.3	16	14

Content of phosphorus and potassium mobile forms decreased by 19 and 26 mg kg^{-1} (respectively) in typical rotation during five years. Losses of phosphorus and potassium by 6 and 8 mg kg^{-1} (respectively) were less in green manure rotation, compared to typical.

Comparative evaluation of agrochemical properties of plowing horizon in sod-podzolic soil revealed that accumulation of available nitrogen, mobile phosphorus and potassium (compared with the control treatment) was observed under systematic application of mineral fertilizers in rates of NPK 60 and 90 kg ha^{-1} for eight years. However, the positive effect of all rates of mineral fertilizers on soil absorbing complex was not noted. Process of acidification was enhanced compared with the soil before experiment foundation in both types of crop rotation.

Application of mineral fertilizers did not promoted the conservation of original humus content, that is coincide with the data obtained by Mineev and Shevtsova (1978). At the same time decreased of humus losses was noted due to adding to the soil more stubble and root residues compared with control treatment (no fertilizers).

Mineral and organic fertilizers use with low rates (NPK 30 kg ha^{-1} of FYM and 20 t ha^{-1}) provided conservation of humus, mobile forms of nitrogen, phosphorus, potassium, and has not stopped the process of soil acidification.

FYM application 40 t ha^{-1} slacked the decline process of organic matter and mobile forms of nitrogen, phosphorus and potassium. Increasing of FYM rate up to 60 t ha^{-1} provided stabilization of soil humus content and mobile forms of nitrogen, phosphorus and potassium as well.

Joint application of organic and mineral fertilizers in moderate rates provided humus conservation in typical crop rotation and its accumulation in green manuring rotation. Positive

changes of soil fertility properties are small en bloc, but are statistically significant as influenced by green manuring rotation.
The positive effect of joint application of organic and mineral fertilizers, even in moderate rates, expressed in raising of nitrogen, phosphorus and potassium mobile forms. Edmeades (2003) made the same conclusions as a result of data generalization of long-term experiments with fertilizers application.
FYM 20 t ha^{-1} and NPK 30 kg ha^{-1} application proved insufficient for soil fertility stabilization at the original level in both crop rotations types. Thus, irreversible changes in plowing horizon of sod-podzolic soil and reducing its fertility takes place under arable land irrational use and application of mineral and organic fertilizers low rates.
The stabilization of soil fertility is provided at the original level by FYM 60 t ha^{-1} in combination with NPK 60 kg ha^{-1} application in typical crop rotation, in green manuring rotation – 40 t ha^{-1} of FYM and NPK 60 kg ha^{-1}.
Results of our research works show that stubble and root residues incoming quantity significantly depends on crop rotation type. So, 53.4 t ha^{-1} of plant residues plowed down into the soil formed during rotation without use of fertilizers in green manuring rotation, 17.5 t ha^{-1} higher than in typical crop rotation. The application of mineral and organic fertilizers in moderate rates provided input increase of plant residues organic matter.
Improvement of agrochemical soil properties, humus content raising due to crop rotation and fertilizers promoted increase of soil biological activity and plants nutrients mobilization that had positive affect on yields level of arable crops.
Comparative general evaluation of field eight-course crop rotations revealed advantage of green manuring rotation (table 2).

Table 2. Effect of crop rotation type on average crops productivity and energy-efficiency of crop rotation

Treatment	Productivity, FU ha^{-1} per year			Energy-efficiency coefficient	
	crop rotation		supplements	crop rotation	
	typical	green manuring		typical	green manuring
No fertilizers	1950	2680	730	2.64	3.12
$N_{60}P_{60}K_{60}$	2220	3020	800	2.24	2.64
$N_{90}P_{90}K_{90}$	2340	3090	750	2.12	2.44
FYM 40 t ha^{-1}	2350	3110	760	2.54	3.07
FYM 40 t ha^{-1} + $N_{60}P_{60}K_{60}$	2400	3140	740	2.15	2.59
FYM 60 t ha^{-1} + $N_{90}P_{90}K_{90}$	2530	3050	520	2.02	2.32
FYM 60 t ha^{-1}	2230	2970	740	2.42	2.72
FYM 60 t ha^{-1} + $N_{60}P_{60}K_{60}$	2500	3190	690	2.06	2.48
FYM 60 t ha^{-1} + $N_{90}P_{90}K_{90}$	2400	3060	660	1.82	2.20
LSD_{05} – 250					

Notes:
FU - feed units
LSD - least significant difference

The highest grain crop yields (2.47-3.09 t ha^{-1}) and average crops productivity (2680-3240 FU ha^{-1}) were obtained in green manuring rotation. The positive effect of FYM on grain crops yield observed for all application rates. However, the yields were higher at 10-69 % in green manuring rotation compared with typical. The highest productivity 2530 FU in typical crop

rotation was noted with application of FYM 60 t ha^{-1} and NPK 90 kg ha^{-1}, in green manuring rotation – 3190 FU with application of FYM 40 t ha^{-1} and NPK 60 kg ha^{-1}. Some other treatments (for instance, $N_{90}P_{90}K_{90}$, FYM 40 t ha^{-1}) provided approximately the same productivity, the difference between these treatments has not exceeded LSD_{05} value.

Joint application of organic and mineral fertilizers at moderate rates also promoted crop yields raising. High rates application of organic (60 t ha^{-1}) and mineral (90 kg ha^{-1}) fertilizers caused lodging of crops and as a result, reduced yields due to losses during harvesting.

Conclusion

Irrational use of arable land leads to soil degradation and, as a result, forming low crop yields. The highest negative balance of humus 0.25 t ha^{-1} per year was noted in typical crop rotation.

Stabilization of soil fertility at the original level was achieved under FYM application 60 t ha^{-1} in combination with NPK in typical crop rotation, in rotation with green-manured fallow – with FYM 40 t ha^{-1} and NPK 60 kg ha^{-1}.

Energy inputs were paid off by crop returns in both rotation types. The energy-efficiency coefficient varied from 1.82 to 3.07 depending on treatment.

Rotation with green-manured fallow with FYM application 40 t ha^{-1} and NPK 60 kg ha^{-1} provided average crops productivity 3140 FU ha^{-1}, grain crop yields 3.0 t ha^{-1}, energy-efficiency coefficient 2.59 and increased organic matter accumulation by 0.16% compared to basic level.

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YIELD AND CARRYING CAPACITY OF PEA – BARLEY AGROPHYTOCENOSIS DEPENDING ON SEEDING RATE OF COMPONENTS AND DOSES NITROGENOUS FERTILIZER IN THE MIDDLE PREDURALIE REGION, RUSSIA

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Abstract

Peculiarities of yield capacity formation of pea and barley grain in mixed sowings were studied in various component ratios and nitrogen fertilizer doses in the conditions of Middle Preduralie of Russia. The authors pointed out that on sod-podzolic heavy loamy, highly provided with phosphorus and potassium soil in years favorable for development of spring grain and unfavorable for legumes, the maximum yields of barley 5.36 t/ha were received at component ratio 12.5%+87.5% during sowing and nitrogen application 60 kg/ha. Crop yield in average over the research years was closely related to quantity of productive stems, which increased in average by 19-84 pcs/m^2, as well as to the increase of grain quantity in ear by 0.7-2.0 pieces and mass of 1000 grains by 1.0-6.5g. Application of nitrogen 60 kg/ha leads to sustainable reduction of pea productivity 4.5 times. This is due to a decline in all indicators of productivity. When applying nitrogen in a dose of 60 kg/ha, total nitrogen contents in barley leaves in the shooting phase reached an optimal level 4.36-4.43%. A direct relationship between the content of total nitrogen in leaves and grains of barley was revealed. Application of nitrogen 60 kg/ha provides crude protein content in barley 14.8-15.0%, and enables coarse grains with the digestible protein 105 g/fodder unit. The content of available energy in mixture grain is higher than 11 MJ/kg. Maximum energy 60.7 GJ/ha and protein crop productivity 646 kg/ha was observed at the component ratio 12.5% +87.5% and application of nitrogen under pre-sowing cultivation in a dose of 60 kg/ha.

Keywords: *pea, barley, mixed sowing, seeding rate, yield capacity.*

Introduction

At the moment increase of fodder production, amelioration of their quality and power ratio is the most important aim of the middle Preduralie regions' agriculture, because developing of cattle breeding and increase its productivity does not raise not only because of fodder shortage, but also because of protein unbalance, which became the reason of fodder considerable overspend and increased spend per unit of animal product. The main source of feed protein for animal is plant fodder. In connection with this the important requirement is liquidation of protein malnutrition and the rise of crude protein content in grain till 14 % and also increase the available energy till 11mJ per 1 kg of dry basis is the rise of fodder quality which is impossible without nitrogenous nutrition correct regulation of legume- grass compounds (Golovnya, Razumeiko, 2012; Shpakov, 2008; Proshkin, 2012).

As a consequence the great significance consists of scientifically based research of fodder deficit methods reducing which has well- balanced protein. One of the important things is organization of adaptive fodder production on the basis of highly productive mixed agrophytocenosis creation. This agrophytocenosis of cultivation was made by trial and error which uses more complete bioclimatic recourses of the region and provides acceptance of high quality fodder combined with recourse-saving and ecofriendly technologies of their cultivation.

The goal of the research is standard optimization of seeding, dose, methods of nitrogen fertilizer application for getting crop yield of pee-barley compound at the level of 4 t/ha with

the content of available energy at least 11 mJ/kg and prosperity of fodder unit digestible protein has at least 105g.
The following aims determined the next steps:
To elicit specialty crop yield seeds formation of pee-barley compound and its component depending on their correlation in the period of seeding and also to elicit the dose and method of nitrogen fertilizer application.
To determine the content of total nitrogen in barley leaves and estimates its connection with the quality of grain.
To make an agro-energy estimation of studying cultivation methods.

Material and methods

Experimental work was realized in 2011-2013 on the trial field of Perm State Agricultural Academy (Russia). The research was made on the sod-podzolic heavy loamy soil with average grade of organic matter (2.2 – 2.9 %), high content of potassium (138 – 150 mg/kg of soil) and phosphorus (293 – 398 mg/kg of soil), with average grade of ammoniac nitrogen (19.5 – 26.2 mg/kg of soil) and nitrate nitrogen (2.7 – 4.1 mg/kg of soil) which is typical for the middle Preduralie region (Mudrykh, Aleshin, 2011).
We applied common methods of establishment and held trail realization during the period of the research (Dospekhov, 1985; Fedin, 1985).
Object of research: common barley “Sonet”, field pea “Gubernator”. Experimental design – Factor A – seeding rate (components correlation (pea + barley) in the period of seeding, % from norm dropping in pure form): A1 – 25 + 75, A2 – 12.5 + 87.5; Factor B – method of nitrogen application: B1 – pre-plant, B2 – radical, B3 – foliage; Factor C – dose of nitrogen, kg/ha: C1 – 0, C2 – 15, C3 – 30, C4 – 45, C5 – 60. Disposition of variants is systematical with help of split-plot method and quadruple tier. Floor area of field is 75 m^2, accountable is 48 m^2. We used drill mixed planting method.
In our trial we used technology of pea- barley agrophytocenosis cultivating which is common for this region (Akmanaev, Zubarev, Eliseev, Renev, 2012). Fore-crop was winter wheat. The main cultivation of soil contained primary tillage LDG – 15B and plowing on the full depth enrichment horizon (PLN 4-35). In spring we made dragging of fell – plowed field BZTS 1.0 into two wheel-track with the following pre-plant wheel-track cultivation KPS-4.0+BZTS-1.0 at depth of 6 - 8 cm. Phosphorous and potassium fertilizers are used in dose of $P_{42}K_{104}$ which cover removal of food compounds with barley crop yield 4t/ha. They were applied under the pre-plant cultivation with spreader L – 116. Nitrogenous fertilizer in forms of calurea is used according to experimental design. The fertilizer was applied by hands under cultivation before seeding. The root application was made in phase of three leaves in barley with seed drill SZ – 3.6. On the stage of barley tillering was made foliar application as plant food with shoulder sprayer of 10% calurea solution. Norms of seeding of culture in pure sowing: pea 1.2 mio, barley 5.5 mio viable seeds per hectare. Planting method of components is mixed with help of row drill SSNP – 16. The depth of seed placement is 4 -5 cm. The single – phase harvesting with combine SK – 5M – 1 at the end of barley yellowing. The crop yield was reached 100% of purity and 14% of humidity.
The result of weather conditions’ evaluation in the period of the experiment shows that it was not typical as to distribution of precipitations and heat supply infrastructure. There was warm weather in May and June. The temperature was about +11.9 и +17.6 C^o, it means that it was higher than long-time average annual level. Also there was enough moisture content. The number of precipitations was 48 – 91 mm, which composed with 89 – 136 % from long-time average annual level.
Such conditions contribute the fast plants growing, especially for the barley in phase of tillering. There was very hot weather in July. The average temperature was +19.8C^o,

moreover there was the rise of the first and second decay till 25-30C^{o}. There was light precipitation (52% from long-time average annual level) which led to plant inhabitation such as pea, barley and reduction of their survival rate especially on the back of the rise nitrogen doses. This situation connected with well developing of nitrogen in phase of ear formation.

Results and discussion

It was defined that crop yield of pea-barley compound formed generally due to cereal component. The part of cereal component in harvest reached 95% (table 1).

We defined that the maximum of grain crop yield compound 5.36 kg/ha was reached with ratio of 12.5+87.5%, pre-plant of nitrogen application in quantity of 60 ha/kg, which is more 0.9 t/ha, than with ratio of 25+75% for 3-year average.

Pea-barley agrophytocenosis crop yield on average for the period of research connected primarily with expansion in the number of correlation barley yielding footstalk to gathering at an average 19 – 84 pcs/m^2, expansion in the number of grains is 0.7 – 2.0 pcs and mass of 1000 grains is 1.0 – 6.5 g. Using nitrogen fertilizer did not depend on the elements of pea crop yield structure, however there is a stable tendency of pea production cropper loss by means of beans quantity reduction on the plant to 0.4 – 1.4 pcs, the quantity of seeds in bean to 0.5 – 1.5 pcs and mass 1000 seeds to 7. – 49.7 g.

Table 1. Crop yield grains of pea-barley compound, t/ha (the average from 2011 to 2013)

Equivalence ratio, % (A)	Dose of nitrogen, kg/ha (C)	Method of application (B)			The average of AC	The average of BC			The average of C
		pre-plant	radical	foliage		pre-plant	radical	foliage	
25+75	0	3.15	3.02	3.12	3.10	3.35	3.29	3.37	3.35
	15	3.66	3.15	3.67	3.49	3.88	3.31	3.86	3.68
	30	3.89	3.41	3.97	3.76	4.17	3.73	4.15	4.01
	45	4.31	3.77	4.13	4.07	4.61	4.01	4.32	4.31
	60	4.46	3.94	4.12	4.17	4.91	4.32	4.51	4.57
The average of *A1B*		***3.89***	***3.46***	***3.80***	***3.72***				
12.5+87.5	0	3.55	3.56	3.62	3.58				
	15	4.09	3.47	4.05	3.87				
	30	4.44	4.04	4.33	4.27				
	45	4.90	4.25	4.51	4.55				
	60	5.36	4.70	4.89	4.98				
The average of *A2B*		***4.47***	***4.00***	***4.28***	***4.25***				
The average of B		4.18	3.73	4.04					
LSD05 r.d.F.A		0.65			LSD05 m.e.F.A		0.17		
LSD05 r.d.F.B		F<F05			LSD05 m.e.F.B		F<F05		
LSD05 r.d.F.C		0.32			LSD05 m.e.F.C		0.13		

Every year in the period of research low crop yield of pea was determined by weak saving intensity of dry basis. The proportion of bean component in common biomass of compound to

barley milk stage did not exceed 3.5%. The quantity of active tuber on the pea root consists of 18 pcs per plant. The duration of their activities is 47 days.
Content of total nitrogen in barley leaves depends on method of application and dose of nitrogen (table 2). This content accomplishes the optimal level only after pre-plant of nitrogen application in amount of 60 kg/ha which consists 4.36-4/43%.
Low efficiency of pre-plant and foliar application appeared because the plant needed the nitrogen fertilizer in earlier period of growth and developing. Also the plant needed the period of realization foliage application. We observed the dry weather in all periods of research.
We revealed straight close relationship between containing of total nitrogen in leaves and grains of barley. The dose of nitrogen essentially influence on crude protein containing in barley grains. The maximum quantity was fixed after application of nitrogen in amounts of 6-kg/ha, on average 14.19%, which is more than other study amounts on 0.87 – 3.65%. As a result for getting fodder grain compound and barley with content of crude protein 14.8 – 15.0% it is necessary to apply nitrogen under pre-plant cultivation in amounts of 60 kg/ha minimum.

Table 2. Nitrogen contained in barley leaves in the beginning of the stage of stem elongation, % (average for 2011 – 2013)

Equivalence ratio, % (A)	Dose of nitrogen, kg/ha (C)	Method of application (B)			The average of AC	The average of BC			The average of C
		pre-planting	radical	foliage		pre-planting	radical	foliage	
25+75	0	1.55	1.69	1.53	1.59	1.54	1.58	1.60	1.57
	15	2.19	1.69	1.68	1.85	2.22	1.68	1.69	1.86
	30	2.79	1.88	1.90	2.19	2.90	1.90	1.92	2.24
	45	3.59	2.21	2.22	2.67	3.57	2.23	2.28	2.69
	60	4.43	2.74	2.74	3.30	4.40	2.80	2.74	3.31
The average of ***A1B***		***2.91***	***2.04***	***2.01***	***2.32***				
12.5+87.5	0	1.54	1.65	1.64	1.61				
	15	2.24	1.66	1.70	1.87				
	30	3.00	1.92	1.93	2.28				
	45	3.56	2.26	2.28	2.70				
	60	4.36	2.87	2.74	3.32				
The average of ***A2B***		***2.94***	***2.07***	***2.06***	***2.36***				
The average of B		2.93	2.06	2.04					

LSD05 r.d.F..A	F<F05	LSD05 m.e.F.A	F<F05
LSD05 r.d.F.B	0.72	LSD05 m.e.F.B	0.23
LSD05 r.d.F..C	0.27	LSD05 m.e.F.C	0.11

High concentration of crude protein in grains ameliorates its feeding value and provides higher digestibility. Calculations shows that compound of feeder grain reached the standard

prosperity of digestible protein to 105 gram per fodder unit. It can be possible only with 60 kg/ha dose of nitrogen fertilizer under pre-plant cultivation (table 3).
Contain of available energy in pea – barley compound grains reached 1.3 mJ/kg and did not depend on correlation of components and approach of nitrogen application. Compound grains without content nitrogen contain less available energy 0.1 mJ/kg. Consequently, it is important to apply the nitrogen under the pre-plant cultivation in amounts of 60 kg/ha for getting barley and pea-barley compound feeding grain in which there will be well balanced protein and contain minimum 11mJ/kg of available energy if the part of pea compound in the harvest is less than 5 %.
Energy output with harvest depends more on quantity and less on the quality. The maximum output of available energy in range of 60.7 gigajoule per hectare. We have got the next correlation of the components 12.5+87.5% and apply of nitrogen under pre-plant cultivation in range of 60 kg/ha however we fixed the maximum of protein seeding productivity on the level of 646 kg/ha.

Table 3. Content of digest protein in pea-barley grain compound depending on components correlation by seeding, method of applying and doses of nitrogen fertilizer, gram per feeding unit (the average to 2011-2013)

Correlation, % (A)	Dose, kg/he (C)	Method of application (B)			The average of AC	The average of BC		
		pre-plant	radical	foliage		pre-plant	radical	foliage
25+75	0	75	75	78	76	74	76	79
	15	84	82	83	83	83	83	84
	30	87	88	90	89	88	89	92
	45	96	95	93	95	95	93	95
	60	105	96	97	99	105	97	98
The average of *A1B*		***89***	***87***	***88***	***88***			
12.5+87.5	0	73	76	79	76			
	15	82	83	84	83			
	30	89	90	93	91			
	45	94	91	97	94			
	60	105	98	99	101			
The average of *A2B*		***89***	***88***	***90***	***89***			
The average of B		89	87	89				

LSD05 r.d.F.A	F<F05	LSD05 m.e.F.A	F<F05
LSD05 r.d.F.B	7	LSD05 m.e.F.B	1
LSD05 r.d.F.C	4	LSD05 m.e.F.C	1

Conclusions

The pea-barley compound with component ratio under seeding 12.5+87.5% is forming crop yield of grains primary due to cereal component. The part in harvest consist of 95% on the sod-podzolic heavy loamy soil of the middle Preduralie region on the first humid and the second arid period of vegetation. The crop yield growth determined the density increase of productive crop 84 pcs/m^2, productivity of ear 0.24 g.

Content of total nitrogen in barley leaves depends on application methods and dose of nitrogen. The optimal level will be reached only with help of pre-plant application of nitrogen in amount of 60 kg/ha and will consist 4.36 – 4.43 % of stem elongation.
Peas and barley mixture sowing with correlation 12.5+87.5% with pre-sowing application of nitrogen dose 60 kg/ha provides crude protein content in barley grain 14.8-15.0 %. It allows getting a fodder with digestible protein level of 105 gram per fodder unit with available energy content in grain of mixture more than 11 MJ/kg, with a maximum output of available energy - 60.7 GJ/ha and collecting digestible protein 646 kg/ha.

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BREEDING AND PROPAGATION OF GARDEN ROSES IN NIKITA BOTANICAL GARDENS

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Abstract

One of the oldest selective establishments in Russia is Nikita Botanical Garden (NBG), where in 1824 it started to select abundant and multi-blooming garden rose varieties, tolerant to climate of Southern Russia. Over 300 domestic sorts of 7 garden groups (Floribunda, Grandiflora, Hybrid Tea, Kordesii, Large-flowered Climber, Miniature, Shrub) were bred here applying various methods including a new system of complex selection of garden roses.
Reproduction of this assortment is carried out in 3 nurseries of NBG, located in different soil and climatic belts of the Crimea: Steppe, South Coastal and close to Sivash lake zones. Capacity to propagate by method of grafting on a seedling stock *R. canina* L. and green and ligneous cutting was investigated in terms of the research. The following was determined: the most favorable periods for cutting and grafting within different zones of the Crimea and methods of cuttings establishment and their growing. Container method for growing cuttings of garden roses, which favors a high seedling output (up to 91-97%), was developed.
Key words*:* *variety, garden roses, breeding, vegetative propagation, cuttings*

Introduction

NBG is one of the oldest breeding institutions of Russia, where in 1824 breeding of garden roses was initiated for the first time. Breeding researches were aimed at breeding of abundant and multi-blooming cultivars, tolerant to hot southern regions of Russia characterized by high air and soil aridity. Over 300 domestic cultivars of 7 garden groups (Floribunda, Grandiflora, Hybrid Tea, Kordesii, Large-flowered Climber, Miniature, Shrub) (Klymenko et al., 2012; Klymenko, 2012) were bred for 200 years applying different selective methods (hybridization, clonal selection and experimental mutagenesis). System of complex breeding of garden roses was developed and adopted to stimulate breeding of garden roses. This system permitted to extend spectrum of form-building processes of garden roses and considerably shorten selection terms of roses while breeding high-ornamental cultivars with transgression of the following parameters: remontantness and disease-resistance (Klymenko, 2012).
Domestic assortment of garden roses differs either by selection methods or by applying distant hybridization for various wild cultivars, what effected on these sorts capacity to propagate.
Moreover in different climatic zones of the Crimea quantitative and qualitative differences of seedlings took place. Being cultivated in these regions they had different degree of frost-resistance.
That`s why objective of this research was to develop and determine the optimal propagation methods and terms of domestic rose assortment in different climatic zones of the Crimea.

Materials and methods

Objects of this study were 350 garden rose cultivars and hybrid forms of domestic and foreign selections at collection plot NBG (Fig.1). They were investigated applying generally accepted methods for study, assessment and propagation of rose varieties (Bylov, 1971; Bylov, Nazarenko, 1968; Vasil´eva O.Y. 2006.; Vasil´eva O.Y. 2008.; Vasil´eva O.Y. 2009; Ambros Ye.V., Vasil´eva O.Y 2013; Uljanov et al., 1987). Green and ligneous cuttings of 2-3 or 4-5 buds were prepared with foliage conservation for further cutting. To intensify establishment of green cuttings they were treated by growth regulators in the following solutions: beta-indolebutyric acid (IBA) with concentration of 25-50 mg/l or beta-indoleacetic acid (IAA, heteroauxin) 50-100 mg/ or alfa-naphthylacetic acid (NAA) 12-25 mg/l. After treating green cuttings were planted into beds equipped by arched film cover and fog-forming system. During the period before root emergence (the first 15 days after cuttings were planted for establishment) fog-forming system is used since 9:00 till 21:00 with interval of 5 minute, water dispersion lasts 12-15 second. In phase of mass establishment (15-25 days after cuttings were planted) fog-forming system works keeping the previous regime. During the phase of total establishment (25-35 days after cuttings were planted) fog-forming system works since 10:00 till 19:00 with 5 minute interval, water dispersion lasts 7-8 second. In 35-40 days cuttings were planted for establishment, time of fog-forming system operation shortens 1 hour every other day. Then before total establishment of cuttings they are watered 1-2 times a week applying water dispersion for 30-40 minute. After cuttings were totally established film cover is taken down, abscissed leaves are removed and mineral fertilizers are used for extra nutrition.

Results and discussion

Using of different breeding methods gave us possibility to obtain assessment of garden roses characterized the by resistance to fungal diseases being cultivated under conditions of South Coast of the Crimea (Fig. 1). Majority of bred varieties of garden groups Floribunda, Grandiflora, Hybrid Tea, Miniature, are notable for not only high ornamental characteristics, but repeated, remontant and continuous blooming till 200 days (Fig. 2).

Propagation of domestic rose varieties is carried out in 3 nurseries of NBG, located in different soil and climatic belts of the Crimea: in north regions of the Crimea - Steppe and close to Sivash lake zones, and south region – South Coast of the Crimea. It was determined that majority of modern garden rose cultivars, based on warm-requiring varieties from the South East Asia being cultivated in north regions of the Crimea are more frost-resistant if to graft them on the local seedling stock, but not to establish scion-rooted plants as a result of cutting. Thereby for Steppe and close to the Sivash lake zones rose propagation on the seedling stock is more preferable, but for South Coast of the Crimea cutting is appropriate as well.

During industrial cultivation capacity of garden roses to different ways of vegetative propagation was investigated and it was found out for some cultivars cutting is the main way to product planting stock, while combination with grafting can be successful for others. According to biological peculiarities of cultivars and demands of economics both propagation ways complementing each other provide high efficiency in production of rose seedlings. Especially high percentage of establishment due to cutting method was marked out for cultivars of the following garden groups: Floribunda, Large-flowered Climber, Miniature.

According to study results method of green cutting is the most prospective method of garden rose propagation on South Coast of the Crimea. This method assumes establishment of cuttings in special beds equipped by artificial fog-system. Special substrate is used for cuttings establishment. It must be quite water and air-penetrating with heat capacity, free of seeds and weeds, pests and agents of diseases. This substrate should consist of two layers:

bottom layer of 15-20 cm thick is a nutrient solution (humus, peat and sand or upper peat, valley peat and pearlite in equal shares) and the upper layer (2-3 cm) is sand.
During research the following was determined: optimal periods of cutting within Steppe Crimea and South Coast of the Crimea, methods of cuttings preparation and treatment by growth regulators, optimal modes of operation for fog-forming system depending on a stage of cutting establishment. According to research data optimal term for green cutting on South Coast of the Crimea is a period (the first decade of May – the first decade of June) when rose shoots are half-lignificated with the phase of budding (phase of coloured bud), and during the mass blooming. After necessary agrotechnical treatment during summer and innovations forming on cut stools, they are used for repeated green cutting in the first decade of August.


Klimentina


Korallovyj Sjurpriz


Sedaja Dama


Jim


Pestraja Fantazia


Nikitsky Yubiley


Polka – Babochka


Kracnyj Majak

Figure 1. Varieties of gardens' roses breeding of NBG

Under conditions of north zone of the Crimea optimal period for green cutting is June. Cuttings can consists of 2-3 or 4-5 buds, leaves are preserved. Cuts of grafts are treated by special powder containing growth regulators. After that green grafts are planted into beds equipped by artificial fog-system and film covering, which is removed in 30-40 days.
Later when cuttings established, mineral fertilizer is added (50g/1 m^2). The following dosing (nitrogen fertilizer) is in 3 weeks, after 3-4 weeks - potash fertilizer. Established cuttings are grown till standard size to produce scion-rooted seedlings of garden roses in the field

conditions for realization. Container method for growing rose established cuttings, which favors a high seedling output (91-97%), was developed in terms of this investigation.
Propagation of garden roses by ligneous cuttings in standard beds is carried out in autumn – September, beginning of October – either in north or in South Coastal zones of the Crimea. Established grafts are grown under field conditions or in containers during the next year and realized.

Figure 2. Long-flowering varieties of garden roses bred in NBG

It was determined that optimal period for grafting on seedling stock of *R. canina* L. in north regions of the Crimea is last decade of August, while on South Coast of the Crimea – the first decade of September. Realization occurs the next autumn.

Conclusions

System of complex breeeding of garden roses developed in NBG favored breeding of more than 300 highly ornamental cultivars of garden roses, which are propagated by two ways: cutting and grafting on stock seedling. It was determined that in north regions of the Crimea cultivation of garden rose is more preferable by grafting on stock-seedling, while for south regions green and ligneous cutting is comfortable as well.

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FLOWERING AND POLLEN GERMINATION OF SOME PEAR CULTIVARS

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Abstract

In the five economically most important cultivars of pears grown in Europe (‘Butirra Precoce Morettini’, ‘Carmen’, ‘Williams’, ‘Conference’ and ‘Abate Fetel’) flowering, pollen germination and pollen tube length were examined in 2012 and 2013 on Radmilovac locality. Pear flowering occurred in the second decade and at the beginning of the third decade of April. ‘Carmen’ cultivar was characterized by the earliest, and the ‘Williams’ cultivar the latest beginning of flowering. Flowering lasted on average 9.4 days and ranged from 8.5 to 11.0 days depending on the cultivar. These examined pear cultivars were statistically very different in terms of pollen germination and pollen tube length. Pollen germination ranged from 42.75% in ‘Williams’ cultivar to 73.54% in ‘Abate Fetel’ cultivar. The lowest length of pollen tubes was found in ‘Butirra Precoce Morettini’ cultivar (644.71 μm), and the highest in ‘Carmen’ (1068.04 μm) and ‘Abate Fetel’ (1009.35 μm). ‘Abate Fetel’ and ‘Conference’ cultivars which showed the highest pollen germination can be recommended as a potentially good pollenisers in new commercial plantations of pear.

Key words: *Pyrus communis, flowering, pollen germination, pollen tube length.*

Introduction

After the grapevine and apple, pear is the third most important continental fruit species that are grown in the world. Production of pears in the world is constantly increasing (Wojciechowski and Antkowiak, 2009). Although there are many cultivars of pears, world production is based on only a few cultivars, such as ‘Williams’, ‘Conference’, ‘Passe Crassane’, ‘Docteur Jules Guyot’, ‘Beurré Anjou’ and ‘Beurré Bosc’ (Bell, 1991; Deckers and Schoofs, 2008).

The main objectives of pear breeding are creating of cultivars with good fruit quality that can long be kept, high and regular productivity, as well as the development of cultivars resistant to climatic factors (frost and drought) and to disease pests (Milatović, 2009).

For good fruit set and achieve high yields, it is necessary to conduct a successful pollination and fertilization. These processes depend on more factors: the success of transfer of pollen, its quality, structure of stigma and style, pollen germination and pollen tube growth, the emergence of incompatibility and effective pollination period (Stösser et al., 1996). One of the very important factors in these processes is the quality of pollen, which varies between individual cultivars within one species. In addition, the quality of pollen depends on the time of taking, methods of collecting and storing conditions (Stanley and Linskens, 1974).

Knowledge of pollen quality is very important for breeding and production of pear. For breeders it is very important to know the functional ability of pollen before crossing, and for producers because of the choice of appropriate pollenisers when planting of pear orchards. This is especially significant at pear, considering to it, that in production beside diploid are cultivated a triploid cultivars, which have low pollen germination and can no used as pollenisers.

Therefore, the aim of this study was to examine phenophase of flowering, pollen germination and pollen tube length, in order to the cultivars with the highest pollen germination are recommended as a potentially good pollenisers when planting of pear orchards and choice of parents for breeding work.

Material and methods

Investigations were carried out at the experimental field "Radmilovac" Faculty of Agriculture, University of Belgrade (Serbia). The subject of this research was five cultivars of pear: 'Butirra Precoce Morettini', 'Carmen', 'Williams', 'Conference' and 'Abate Fetel'. The rootstock is quince BA 29, training system is 'Slender Spindle' and planting distance is 3.5 x 1.0 m. Researches were carried out during 2012 and 2013.

In the mentioned cultivars, phenophase of flowering, pollen germination and pollen tube length were investigated. Phenophase of flowering was monitored by the recommendations of the international working group for pollination (Wertheim, 1996). Beginning of flowering is registered as 10% of open flowers, full of flowering when 80% of flowers open, and the end of flowering, when it fell 90% petal. The duration of flowering is given as the number of days from the beginning to the end of flowering and abundance of flowering estimates from 0 to 5.

For examination of pollen germination branches with flower buds in the 'balloon' phase were taken and carried to the laboratory. In order to collect pollen from the flower buds anthers were isolated in Petri dishes. They are stored at room temperature (20±2ºC) for 24-48 h to dry and to release the pollen. Then, the pollen of each cultivar was sown with fine brushes in Petri dishes (9 cm diameter) on the previously prepared nutrient medium consisting of 15% sucrose and 0.7% agar-agar.

Petri dishes with a sowed pollen were observed under light microscope 'Leica DM LS' (Leica Microsystems, Wetzlar, Germany), for counting of germinated pollen grains. The experiment was done in three repetitions, each including at least 300 pollen grains. Pollen is considered as germinated if the length of pollen tube was larger than the diameter of the pollen grain.

Pollen tube length was measured from images taken under the microscope 'Leica DM LS' using 'Leica IM 1000' program. From each cultivar 60 pollen tubes were measured.

The obtained results were processed statistically with the help two factorial analysis of variance. Percentage data were subjected to arcsin square root transformation before the statistical analysis. Duncan's multiple range test (5% and 1%) was performed for means comparison. Data analysis was performed using the statistical software package 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and discussion

Knowing flowering time of pear cultivars is very important for selecting the most appropriate pollenisers. Pear flowering occurred in the second decade and at the beginning of the third decade of April (Table 1). 'Carmen' cultivar was characterized by the earliest (11 April), and the 'Williams' cultivar the latest beginning of flowering (14 April). Beginning of flowering is very varied by years of research. All studied cultivars were beginning to flowering earlier in 2012 compared to 2013. Beginning of flowering in 2012 was an average of two weeks earlier (05 April) compared to 2013 (19 April). The differences between years can be linked to differences in air temperatures just before the beginning of flowering, which were higher in 2012 compared to 2013. Full of flowering is occurred on average two days after the beginning of flowering and this sub-phases is also registered earliest at cultivar 'Carmen' (12 April), and latest at cultivar 'Williams' (16 April). Consequently, the end of flowering is occurred earliest at cultivar 'Carmen' (20 April), and latest at cultivar 'Williams' (23 April).

Table 1. Flowering of investigated pear cultivars.

Cultivar	Year	Beginning of flowering	Full of flowering	End of flowering	Duration of flowering (days)	Abundance of flowering (0-5)
‘Butirra Precoce Morettini’	2012	05 April	07 April	15 April	15.0	2.0
	2013	19 April	21 April	26 April	7.0	2.0
	Mx	**12 April**	**14 April**	**21 April**	**11.0**	**2.0**
‘Carmen’	2012	04 April	05 April	15 April	11.0	4.0
	2013	18 April	19 April	24 April	6.0	5.0
	Mx	**11 April**	**12 April**	**20 April**	**8.5**	**4.5**
‘Williams’	2012	07 April	09 April	18 April	11.0	3.0
	2013	21 April	23 April	28 April	7.0	3.0
	Mx	**14 April**	**16 April**	**23 April**	**9.0**	**3.0**
‘Conference’	2012	06 April	08 April	17 April	11.0	3.0
	2013	20 April	22 April	27 April	7.0	3.0
	Mx	**13 April**	**15 April**	**22 April**	**9.0**	**3.0**
‘Abate Fetel’	2012	05 April	08 April	16 April	11.0	5.0
	2013	18 April	20 April	26 April	8.0	5.0
	Mx	**12 April**	**14 April**	**21 April**	**9.5**	**5.0**
Year	2012	05 April	07 April	16 April	11.8	3.4
	2013	19 April	21 April	26 April	7.0	3.6
	Mx	**12 April**	**14 April**	**21 April**	**9.4**	**3.5**

Flowering lasted an average of 9.4 days, with a variation between cultivars of 8.5 days (‘Carmen’) to 11.0 days (‘Butirra Precoce Morettini’). Data on the duration of flowering in pear agree with the allegations Mratinić (2000). The duration of flowering was shorter in 2013. The reason is that the flowering of cultivars in 2013 occurred later, when are higher air temperatures, which caused the faster flow of phenophase flowering in this year.

Abundance of flowering is a genetic trait of species ie cultivar of which depends largely on fruit set and yield (Mratinić, 2000). This trait is very varied among the pear cultivars in our experiment. ‘Abate Fetel’ cultivar had the highest, ie maximum score for the abundance of flowering (5.0). In addition to the cultivar ‘Abate Fetel’, high score for abundance of flowering had a ‘Carmen’ cultivar (4.5). On the other hand, the cultivar ‘Butirra Precoce Morettini’ is rated as the worst in terms of the abundance of flowering (2.0).

Test of pollen germination *in vitro* is one of the main indicators of functional pollen viability (Figure 1). The average pollen germination of all cultivars in the two-year period amounted to 53.09% (Table 2). Pollen germination is statistically very significant different among the pear cultivars. The highest pollen germination was found in ‘Abate Fetel’ cultivar (73.54%). Mratinić (2000) points out also for this cultivar that has a good germination of pollen. In addition to the ‘Abate Fetel’ cultivar, high pollen germination was characterized by the ‘Conference’ cultivar (61.79%). On the other hand, pollen germination rate was the lowest in the ‘Williams’ cultivar (42.75%). Stančević (1980) states that the pollen germination of diploid pear cultivars varies from 30% to 100%. Pollen germination of the cultivars tested in our work was at the mentioned interval. Sharafi (2011) found that the pollen germination of some indigenous of pear cultivars in Iran ranged from 28.4% to 80.8%. However, Jovančević (2002) established that the genotypes of wild pear (*Pyrus communis*) are characterized by an extremely high pollen germination (average 94.76%).

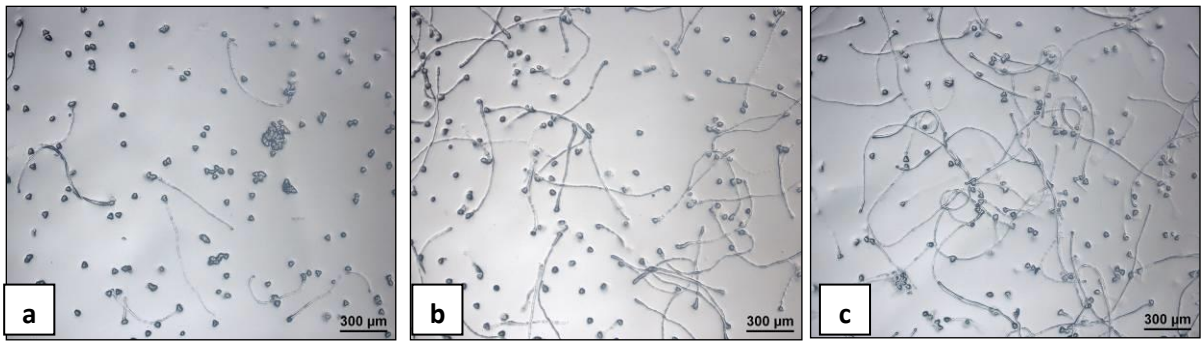

Figure 1. Pollen germination in pear cultivars: a) ‘Carmen’, b) ‘Conference’, c) ‘Abate Fetel’.

Apart among the cultivars, pollen germination is very significantly different between the investigated years. It was on average about 20% higher in 2013 compared to the year 2012. This can be explained by the influence of different temperatures between years at pollen germination, which is in line with the results of Deckers and Porreye (1984). It was found that despite the low, high temperatures during anther development affecting the poorer pollen germination (Pressman et al., 2002).

Table 2. Pollen germination of investigated pear cultivars (%).

Cultivar/Year	2012	2013	**Mx**
‘Butirra Precoce Morettini’	25.95	60.55	**43.25 c**
‘Carmen’	24.63	63.60	**44.12 c**
‘Williams’	24.67	60.83	**42.75 c**
‘Conference’	57.21	66.38	**61.79 b**
‘Abate Fetel’	79.28	67.80	**73.54 a**
Mx	**42.35 b**	**63.83 a**	**53.09**

In addition to pollen germination, pollen tube length is a very significant different between the pear cultivars (Table 3). In the ‘Carmen’ and ‘Abate Fetel’ cultivars was determined the highest of pollen tube length (1068.04 µm and 1009.35 µm). In contrast, the pollen tube length was the lowest in ‘Butirra Precoce Morettini’ cultivar (644.71 µm). The pollen tube lenght in our work was significantly higher than the indigenous pear cultivars which examined by Sharafi (2011) in Iran. This is due to genetic differences between the two groups of cultivars.

Table 3. Pollen tube length of investigated pear cultivars (µm).

Cultivar/Year	2012	2013	**Mx**
‘Butirra Precoce Morettini’	418.85	870.58	**644.71 c**
‘Carmen’	611.15	1524.92	**1068.04 a**
‘Williams’	453.06	1098.36	**775.71 b**
‘Conference’	768.68	1171.81	**970.25 a**
‘Abate Fetel’	913.75	1104.95	**1009.35 a**
Mx	**633.10 b**	**1154.12 a**	**893.61**

Similarly pollen germination, pollen tube length is very significant different between the years of research. It was an average for all cultivars two times higher in 2013 (1154.12 μm) compared to 2012 (633.10 μm).

Conclusion

Based on the research results of flowering, pollen germination and pollen tube length of five the most important pear cultivars in the Belgrade area was determined following:

Pear flowering occurred in the second decade and at the beginning of the third decade of April. ‘Carmen’ cultivar was characterized by the earliest, and the cultivar ‘Williams’ the latest beginning of flowering.

Phenophase of flowering in the cultivars studied in this paper is quite coincided, so according to this criterion all cultivars can be used as mutual pollenisers.

These examined pear cultivars were statistically very different in terms of pollen germination and pollen tube length. ‘Abate Fetel’ and ‘Conference’ cultivars which showed the highest pollen germination can be recommended as a potentially good pollenisers in new commercial plantations of pear.

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POLLEN GERMINATION AND POLLEN TUBE GROWTH *IN VITRO* IN QUINCE CULTIVARS

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Abstract

Pollen germination and pollen tube growth *in vitro* in eight quince cultivars (‘Leskovacka’, ‘Vranjska’, ‘Morava’, ‘Pazardzijska’, ‘Hemus’, ‘Asenica’, ‘Portugal’ and ‘Triumph’) were studied in the three-year period (2010-2012). Agar-plate method with three different concentrations of sucrose (10%, 15% and 20%) was applied. Statistically significant differences between quince cultivars were found in terms of pollen germination and pollen tube length. The highest germination rate was found in 15% sucrose (78.72% in average). Slightly lower germination was found in 20% sucrose (78.52%), while the lowest germination was determined in 10% sucrose (73.76%). Among quince cultivars, the highest germination rates were found in ‘Triumph’ and ‘Asenica’, and the lowest in Leskovacka. Increasing the concentration of sucrose increased the pollen tube length (from 1435.8 μm in 10%, followed by 1790.7 μm in 15% to 2077.5 μm in 20%). Pollen tube length in 20% sucrose concentration ranged from 1770.3 μm (‘Vranjska’) to 2574.4 μm (‘Asenica’). Based on obtained results, the sucrose concentrations of 15% and 20% can be recommended as optimal for pollen germination of quince cultivars.

Key words: *Cydonia oblonga, sucrose, pollen germination, pollen tube length*.

Introduction

Pollen germination and pollen tube growth are the most important characteristics that determine the quality of pollen. The quality of pollen is a very important factor for successful fertilization, fruit set and yield in quince (*Cydonia oblonga* Mill.).

Pollen viability varies between individual cultivars within the same fruit species (Stösser et al., 1996). In addition to the cultivar, pollen viability depends of rootstock on which the cultivar is grafted (Kidman et al., 2014). Besides these factors, pollen viability varies depending of microclimate, locality, character of last year's vegetation, vigour, age, nutrition and health status of fruit trees (Pejkić, 1980). Pollen viability can be affected by the presence of heavy metals: Cd, Cu, Hg and Pb (Gür and Topdemir, 2008; Kiliç et al., 2009), boric acid (Imani et al., 2011; Mondal and Ghanta, 2012; Liu et al., 2013), plant growth regulators (Bolat and Pirlak, 2003; Tosun and Koyuncu, 2007) and the fungicides (Yi et al., 2003; Kargar and Imani, 2011). Also, the quality of pollen depends on the time of taking, methods of collecting (Stanley and Linskens, 1974), the length and mode of its storage (Aslantas and Pirlak, 2002; Perveen and Khan, 2008). The germination of pollen varies depending of temperature (Pirlak, 2002; Sorkheh et al., 2011; Milatović and Nikolić, 2014).

One of the most important factors affecting to the pollen germination is sucrose concentration (Dalkiliç and Mestav, 2011; Sutyemez, 2011). The optimum concentration of sucrose for testing the pollen germination varies between different fruit species and it is ranging from 10 to 20% (Mahanoğlu et al., 1995; Milatović and Nikolić, 2007; Mert, 2009; Dalkiliç and Mestav, 2011; Sutyemez, 2011). However, the concentration of sucrose over 20% may have an inhibitory effect on pollen germination (Bolat and Pirlak, 1999).

Knowledge of pollen functional ability is a very important parameter in fruit breeding for the choice of male parent in hybridization. In addition, this parameter is very significant in the production practices for the selection of appropriate pollenisers when planting of quince orchards, because pollenisers, among other parameters need to be distinguished by good pollen germination.
The aim of this study was to determine the pollen germination and pollen tube length in eight quince cultivars, in order to evaluate suitability of these cultivars as potential pollenisers. Also, testing of nutrient media with different concentrations of sucrose aimed to determine the optimal concentration for testing pollen germination and pollen tube length.

Material and methods

Investigations were carried out in the collection orchard of quince at the Experimental Station 'Radmilovac' of the Faculty of Agriculture in Belgrade during a three-year period (2010-2012). The orchard was established in the spring of 1999, with the planting space of 4.5 x 3 m. The study comprises eight cultivars of quince: 'Leskovacka', 'Vranjska', 'Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph'. The examined cultivars were grafted on the rootstock 'Quince MA'.
For examination of pollen germination branches with flower buds in the 'balloon' phase were taken and carried to the laboratory. In order to collect pollen from the flower buds anthers were isolated in Petri dishes. They are stored at room temperature (20±2ºC) for 24-48 h to dry and to release the pollen. Then, the pollen of each cultivar was sown with fine brushes in Petri dishes (9 cm diameter) on the previously prepared nutrient medium consisting of three different sucrose concentration (10%; 15% and 20%) and 0.7% agar-agar.
Petri dishes with a sowed pollen were observed under light microscope 'Leica DM LS' (Leica Microsystems, Wetzlar, Germany), for counting of germinated pollen grains. The experiment was done in three repetitions, each including at least 300 pollen grains. Pollen is considered as germinated if the length of pollen tube was larger than the diameter of the pollen grain.
Pollen tube length was measured from images taken under the microscope 'Leica DM LS' using 'Leica IM 1000' program. From each cultivar 60 pollen tubes were measured.
The data were statistically analyzed using three-factor analysis of variance (ANOVA). In the results expressed in percentages, the arcsin square-root data transformation was performed. The significance of differences between the mean values was determined using the Tukey's test for significance level $p < 0.05$. Data analysis was performed using the statistical software package STATISTICA, Version 8 (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and discussion

Test of pollen germination *in vitro* is one of the main indicators of pollen functional ability (Figure 1). The average pollen germination for all cultivars in the three-year study period was quite high and amounted to 77.00% (Table 1). It has significantly varied between the examined cultivars and ranged from 65.12% ('Leskovacka') to 84.74% ('Triumph'). Pollen germination at a concentration of 10% sucrose was highest in 'Triumph' cultivar (82.40%) and lowest in 'Leskovacka' cultivar (62.61%). 'Leskovacka' cultivar had lowest pollen germination at sucrose concentrations of 15% (64.62%) and 20% (68.12%), while the 'Asenica' cultivar had highest pollen germination in sucrose concentrations 15% (86.43%) and 20% (87.58%). In addition to 'Asenica' and 'Triumph' cultivars, high pollen germination had 'Pazardzijska' and 'Morava' cultivars, which is consistent with the findings of Stančević (1986) and Stančević (1990). Pollen germination between quince cultivars examined in our work was much less varied in relation to the one established by Sharafi (2011a) in Iran, in which the pollen germination ranged from 45.4% to 82.3%. This is due to genetic differences between tested cultivars.

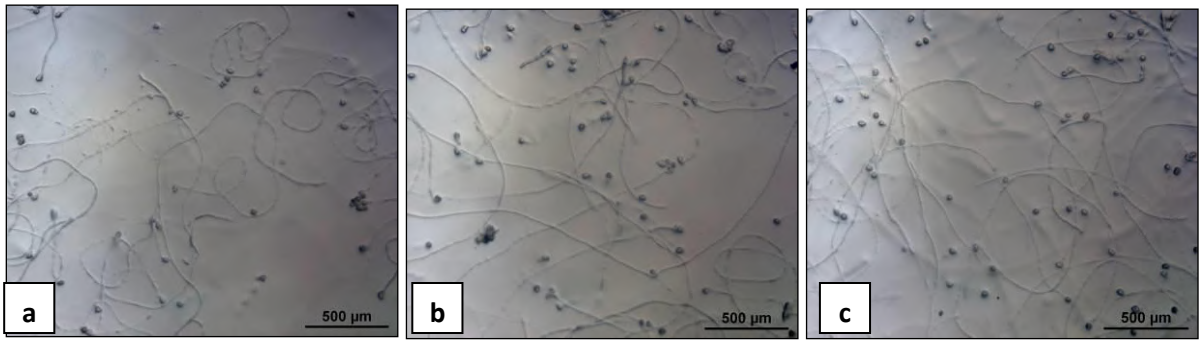

Figure 1. Pollen germination of Triumph cultivar at three different sucrose concentrations: a) 10%, b) 15%, c) 20%.

In addition to the cultivars, pollen germination differed significantly between investigated concentrations of sucrose, which is in accordance with the results Dalkiliç and Mestav (2011). The highest germination rate was found in 15% sucrose (78.72% in average). Slightly lower germination was found in 20% sucrose (78.52%), while the lowest germination was determined in 10% sucrose (73.76%). These results partially agree with those obtained by Çetin and Soylu (2006), where the highest pollen germination in quince cultivars was found in 10% and 15% sucrose solution. Pollen germination rate also varied depending on the concentration of sucrose in the Japanese pear (Okusaka and Hiratsuka, 2009), apricot (Mahanoğlu et al., 1995; Milatović and Nikolić, 2007; Asma, 2008), sweet and sour cherry (Bolat and Pirlak, 1999; Sutyemez, 2011) and walnut (Mert, 2009).

Table 1. Pollen germination (%) of investigated quince cultivars (average 2010-2012).

Cultivar/Year		Sucrose concentration			Mx
		10%	15%	20%	
‘Leskovacka’		62.61	64.62	68.12	**65.12 d**
‘Vranjska’		68.75	71.86	75.45	**72.02 c**
‘Morava’		74.41	82.60	80.61	**79.21 b**
‘Pazardzijska’		74.14	80.76	78.75	**77.88 b**
‘Hemus’		76.76	80.47	77.27	**78.17 b**
‘Asenica’		79.38	86.43	87.58	**84.46 a**
‘Portugal’		71.64	77.22	74.36	**74.41 c**
‘Triumph’		82.40	85.81	86.02	**84.74 a**
Year	2010	80.26	83.13	83.00	**82.13 b**
	2011	80.61	85.79	84.69	**83.70 a**
	2012	60.41	67.24	67.87	**65.17 c**
Sucrose concentration		**73.76 b**	**78.72 a**	**78.52 a**	**77.00**

The pollen germination in our study was significantly different between years, but it was a more balanced in the first two years, amounting to over 82%. However, it was about 15-20% lower in 2012 and amounted to 65.17%. It can be connectted with the influence of low temperatures on the pollen germination in this year.

Pollen tube length was significantly different among quince cultivars, sucrose concentrations and years of study (Table 2), which is in line with the results Milatović and Nikolić (2007). An average length for all cultivars was 1768.0 µm, and it varied between cultivars from 1577.5 µm (‘Hemus’) to 2015.8 µm (‘Asenica’). At a sucrose concentration of 10%, pollen

tube length ranged from 1236.1 μm (‘Hemus’) to 1674.2 μm (‘Portugal’). ‘Hemus’ cultivar had shortest pollen tubes (1411.9 μm) in 15% sucrose solution, and the longest pollen tube lenght had ‘Asenica’ cultivar (2072.0 μm). At a sucrose concentration of 20% lowest pollen tube lenght had ‘Vranjska’ cultivar (1770.3 μm), and highest ‘Asenica’ cultivar (2574.4 μm). Pollen tube lenght in our work was significantly higher compared to the results obtained by Sharafi (2011 a, b), at the quince cultivars in Iran, what is result of genetic differences between these two groups of cultivars.

Table 2. Pollen tube length (μm) of investigated quince cultivars (average 2010-2012).

Cultivar/Year		Sucrose concentration			Mx
		10%	15%	20%	
‘Leskovacka’		1543.2	1898.5	2011.9	**1817.9 ad**
‘Vranjska’		1375.0	1797.2	1770.3	**1647.5 bcde**
‘Morava’		1371.5	1593.9	1926.2	**1630.5 cde**
‘Pazardzijska’		1528.0	1839.6	2154.0	**1840.5 ab**
‘Hemus’		1236.1	1411.9	2084.5	**1577.5 e**
‘Asenica’		1401.0	2072.0	2574.4	**2015.8 a**
‘Portugal’		1674.2	1735.2	1957.2	**1788.9 bcd**
‘Triumph’		1357.3	1977.1	2141.5	**1825.3 ac**
Year	2010	1774.0	2121.5	2300.4	**2065.3 b**
	2011	1964.5	2407.4	2798.8	**2390.2 a**
	2012	568.8	843.1	1133.2	**848.4 c**
Sucrose concentration		**1435.8 c**	**1790.7 b**	**2077.5 a**	**1768.0**

Increasing the concentration of sucrose increased the pollen tube length (from 1435.8 μm in 10%, followed by 1790.7 μm in 15% to 2077.5 μm in 20%). This trend was evident in all cultivars except the ‘Vranjska’ cultivar, in which the pollen tube length was the highest at a concentration of 15% sucrose. However, Bolat and Pirlak (1999) were determined the highest pollen tube length at sucrose concentrations of 10% and 15% at some cultivars of apricot, sweet and sour cherry. This indicates that the optimal concentration of sucrose for testing of pollen tube growth depends of fruit species.

Depending on meteorological conditions, the pollen tube length has differed by years. It was the highest in 2011, and the lowest in 2012. The occurrence of late spring frosts in April 2012 reduced the pollen tube length two to three times compared to the previous two years. This effect was present in all tested cultivars, except for ‘Asenica’ cultivar at sucrose concentrations of 15% and 20%.

Conclusion

Based on the results of three year trials, it can be concluded that the pollen germination and pollen tube length differed significantly depending on the cultivar, year and the concentration of sucrose. ‘Triumph’ and ‘Asenica’ cultivars were characterized with highest and ‘Leskovacka’ cultivar with lowest pollen germination. All studied cultivars had high pollen germination, so they can be recommended as a potentially good pollenisers in new commercial plantations of quince. Based on obtained results, the sucrose concentrations of 15% and 20% can be recommended as optimal for pollen germination of quince cultivars.

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EXTRACTION OF NATURAL COLOURANTS FROM THE GRAPESKIN OF CABERNET SAUVIGNON

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Abstract

Recently, various technologies have been developed in order to achieve maximal utilization of by-products of wine production as well as greater productivity. One of the most potential by-products of wine production is grape pomace, which is still very rich source of anthocyanins. It is well known that these natural colourants have high antioxidant activity.

The aim of this research was optimization of anthocyanins extraction from grape skin of black grape variety *Cabernet Sauvignon*. The extraction was carried out with water and water/ethanol in different ratios. The extraction efficacy and kinetics as well as the application of ultrasound (40 kHz) were examined.

The content of total anthocyanins and total polyphenols were determined by pH differential and the Folin-Ciocalteu method, respectively.

The results indicate a significant influence of ethanol and ultrasound on the anthocyanins extraction efficiency. In addition, the time needed for the extraction maximum was significantly decreased (90 minutes) compared to water extraction. The best results have been achieved with 70% ethanol solution exposed to ultrasound, where a three times higher contents of anthocyanins and total polyphenols were obtained than in water extracts.

The grape skin of black varieties, especially *Cabernet Sauvignon*, could be important source of natural colourants and bioactive compounds. Furthermore, it should represent replacement for artificial colourants in numerous food products in the current worldwide market.

Key words: *extraction, grapeskin, anthocyanins, colourants, polyphenols*

Introduction

Utilization of by-products from the food industry is an increasing economic and ecological issue. There are trends of developing novel technologies, in order to achieve maximal exploitation of by-products and greater productivity with minimal environmental contamination (Schieber et al., 2001; Galanikis 2012).

Anthocyanins are well known as a valuable bioactive compounds and their addition in food product has a great potentials (Liang et al., 2008; Iriti and Faoro, 2011). Problem with applying of these components in food is low stability during the processing and storage of food or in the gastrointestinal tract. The effectiveness of these components depends on preservation of their stability, bioactivity and bioavailability which are limited by external influences.

In order to obtain the extracts of better quality, different types of extractions and traditional extractions (maceration, with the use of enzymes) are increasingly being replaced by modern, such as ultrasonic extraction. With this type of extraction, which is actually modified by maceration, extraction of anthocyanins and other polyphenolic compounds is improved by using ultrasound. The effect of ultrasound on the extraction of bioactive compounds from

different plant material is attributed to: cavitation effects (affect the destruction of the cell wall), hydration of material, particle size reduction, and the acceleration of the mass transfer.
In our research authors compared the efficacy and extraction kinetics of water and water/ethanol extractions with and without the application of ultrasound (40 kHz) during 360 minutes.

Material and Methods

In this study grapeskin of black grape variety *Cabernet Sauvignon* was used. This grape variety was grown at the experimental school estate of Faculty of Agriculture – "Radmilovac", Serbia.
Chemicals: Potassium chloride and ethanol were procured from Centrohem (Belgrade, Serbia). Citric acid and sodium hydrogen phosphate were purchased from Hemos (Belgrade, Serbia). Potassium dihydrogen phosphate was provided by Fisher Chemical (Lougborogh, UK). Gallic acid, Folin-Ciocalteu's phenol reagent, ammonium hydroxide, hydrochloric acid, sodium acetate trihydrate and sodium carbonate were purchased from Merck (Darmstadt, Germany).

Extraction

Milled grapeskin was extracted with water, 50 %v/v and 70 %v/v ethanol solutions during 360 minutes. The samples were extracted by shaking (200 rev/min) with and without the application of ultrasound. The ultrasonic bath (Elma) with frequency 40 kHz was used. Ethanol was chosen as the solvent based on its environmentally friendly characteristics and its ability to enhance the extraction of phenolic constituents and anthocyanins from grapeskins, respectively, compared to mono-component systems (Spigno et al., 2007). Pinelo et al. (2005) also pointed out the advantages of using ethanol instead of methanol and water.

Determination of total anthocyanins

The content of total monomeric anthocyanins was determined by pH differential method that is based on the structural transformation of anthocyanins in change in pH (Lee et al., 2005). The monomeric anthocyanins are subject to structural transformation of the reversible as a function of pH, wherein at pH = 1 are in the form of intensely stained flavilijum cation, and at a pH = 4.5 taking the form of a colorless hemiketal. Anthocyanins' content is determined by measuring the change in absorbance at two different pH values (pH = 1 and pH = 4.5), whereby the difference in absorbance of a pigment at a wavelength of 520 nm is proportional to the concentration of the pigment. The concentration of the monomer is expressed in anthocyanin cyanidin-3-glucoside according to the equivalents of the following formula:

$$\textit{anthocyanin content(cyanidin-3-glucoside equivalents, mg/L)} = A \times MW \times DF \times 10^3 / \mathcal{E} \times l,$$

where $A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established in D; l = pathlength in cm; $\mathcal{E}$ = 26 900 molar extinction coefficient, in $Lmol^{-1}cm^{-1}$, for cyd-3-glu; and 10^3 = factor for conversion from g to mg.

Determination of total polyphenols

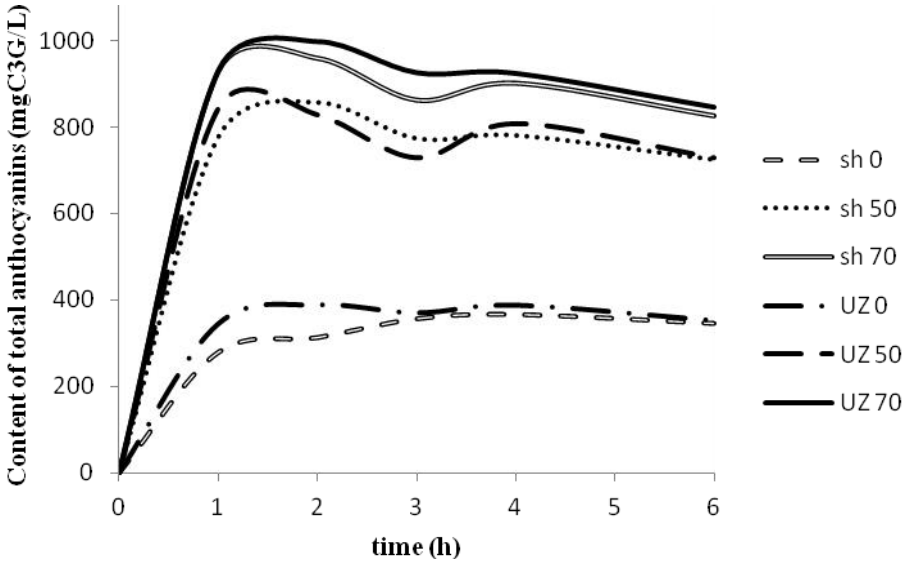
The total phenolic content of samples was determined according to the Folin-Ciocalteu spectrophotometric method by using UV-Vis Double beam spectrophotometer (HALO-DB/2S Dynamica) (Singelton and Rossi, 1965). All samples of extracts were mixed with distilled

water in order to get dilutions of appropriate concentration. 125 µL of the diluted samples and 1.25 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent were mixed and allowed to react for 4 min. One milliliter of saturated sodium carbonate (75 g/L) was added to the mixture and then shaken. After 120 minutes of reaction at room temperature in the dark, the absorbance at 760 nm was determined. The measurement was compared to a calibration line of prepared gallic acid (GA) solution, and the results were expressed as milligrams of gallic acid equivalents per liter of extract (mg GAE/L).

Results and discussion

Determination of total anthocyanins

In this study six different extracts of grape skin were obtained. As can be seen from the Graph 1 the best extraction has been achieved with 70 %v/v ethanol solution exposed to ultrasound.

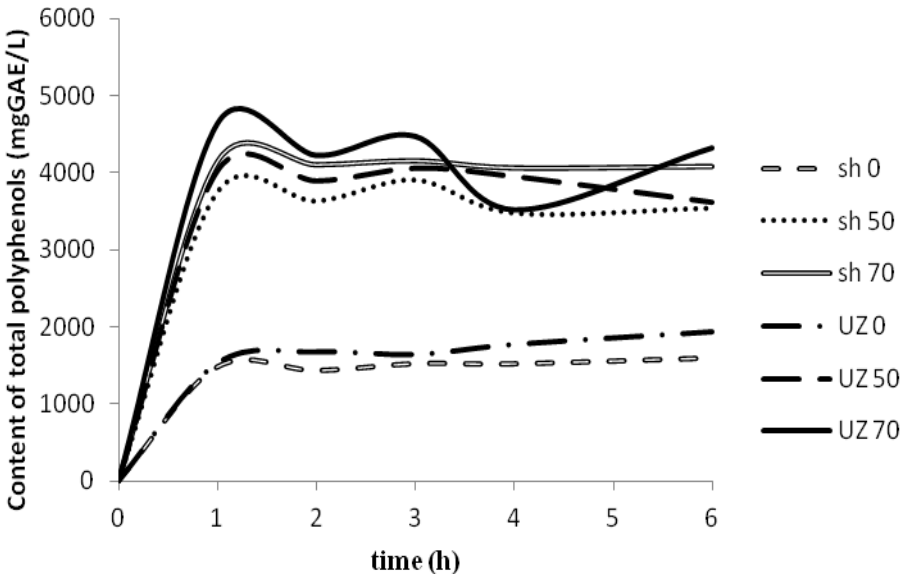


Graph 1. Extraction kinetics of total anthocyanins
(sh-shaking, UZ-ultrasound, 0-water extraction, 50-ethanol/water ratio 50/50, 70- ethanol/water ratio 70/30)

Ultrasound showed better results in each sample (with all three types of solvents), but not significantly different. However, the influence of ethanol was very important. The difference between samples extracted with and without alcohol has been statistically significant (about 900 mg C3G/L compared to 350 mg C3G/L).

Determination of total polyphenols

Addition of ethanol to water significantly improved the extraction rate of total polyphenols (Graph 2). Phenols extracted with different ethanol content in solutions showed similar results, but still the highest result has been achieved with 70 %v/v ethanol solution.



Graph 2. Extraction kinetics of total polyphenols
(sh-shaking, UZ-ultrasound, 0-water extraction, 50-ethanol/water ratio 50/50, 70- ethanol/water ratio 70/30)

The results represented in Corrales et al (2008) and Kalušević et al (2015) studies also confirmed very significant effect of the ultrasound application on grapeskin phenols and anthocyanins extraction in comparison to control extraction.
As can be concluded from both graphs the time needed for the maximum of extraction of polyphenols and anthocyanins has been decreased and achieved in the first 90 minutes of the extraction. Kalušević et al (2015) have presented the same optimum time (90 minutes) for water extraction with ultrasound from Prokupac grapeskin, compared to 180 and 270 minutes in samples without application of the ultrasound.

Conclusions

The extracts of black varieties' grapeskin could be a significant source of natural colourants and bioactive compounds, thus potential additives for some functional products. The potential applications of the ultrasound for bioactive compound extraction must be studied on the basis of the matrix characteristics, solvent choice, liquid–solid ratio, temperature, and time. Due an economical issue is crucial one for the industrial implementation the combination of effective extraction and low-cost raw materials represent and environmental and economical solution in comparison to conventional extraction methods where large amounts of organic solvents and long extraction times are required. The use of the novel processing technologies will reduce food processing wastes and facilitate the production of natural valuable products which will guarantee food sustainability and also, meet consumer demands.

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DIFFERENT ASPECTS OF MODE OF ACTION OF BRASSINOSTEROIDS IN MAIZE

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Abstract

In contrast to the mechanism of action, the mode of actions of certain chemicals in the plants is defined as the totality of the effects that these chemicals cause the effect on metabolism, growth and development of plants. In this sense, we studied the effect of the broad concentration range of 24-epibrassinolide on germination of two maize hybrids (ZP434& ZP704), whereby the monitoring of effect mentioned brassinosteroid phytohormone on germination, and some thermodynamic and biochemical parameters in the seeds during germination. Then we are exposed plants of the maize hybrid (ZP505), grown in different light environments (light vs. shadow) or at different volumes of pot (in which we cultivated plants), to the effects of 24-epibrassinolide or propiconazole (an inhibitor of the synthesis of brassinosteroids). During trial we registered some parameters of growth and photosynthesis. And finally, we conducted a mini field trial treating two maize hybrids (ZP434& ZP341) with the broad range of doses of 24-epibrasinolide, as well as with two doses of propiconazole. We checked the final yield and some characteristics of growth of maize plants in the field, as well as the chemical composition of the grain after harvest. The results are discussed from the perspective of a 24-epibrassinolide effects on maize plants of different ages.

Key words: *mode of action of 24-epibrassinolide, germination, whole plant physiology*

Introduction

Brassinosteroids (BRs) are known to have a crucial role in development of a plants (Clouse and Sasse, 1998). But, the main goal of contemporary investigations of action of these phytohormones are concentrated on mechanisms of their action. In contrast to the mechanism of action, the mode of actions of certain chemicals in the plants is defined as the totality of the effects that a chemicals cause the effect on metabolism, growth and development of plants. In this sense, we studied the effects of 24-epibrassinolide (24-EBL; concentration range: 5.2 X 10^{-7} – 5.2 X 10^{-15} M) on the different maize hybrids in a developmental stages.

Material and methods

Seeds of two maize (*Z. mays* L.) hybrids (ZP434& ZP704) were germinated 7 days in a phytotron (light/dark period: 12/12 h, 30/20 ^{0}C; PAR~100 μmol $m^{-2}s^{-1}$), according ISTA rules. The seeds were germinate on solutions of 24-EBL („Epin-Extra“®, Russia) of different concentrations (5.2 X 10^{-7} - 5.2 X 10^{-15} mol, and control). After that procedure we checked the percentage of germinated seeds, and absolute and relative fresh weight of young plant parts (plumule, radicle& rest of seed) and weights after drying them at 60, 105 and 130 ^{0}C. Measured values used for calculations of thermodinamic parameters (Sun, 2002) of

germination proceses. Photosynthetic pigments are determined according to Lichtenthaler (1987). Samples for sugar determination was prepared, and extracted in a conventional manner, and then quantified by ion chromatography method. Maize plants (ZP505) were grown in half-shade (PARmax≈ 500μmol photon $m^{-2}s^{-1}$) 1 month, in the pots of V=5 l (2/3 so called (s.c.) “5 plants”) and V=11 l (1/3 s.c. “11 plants”), until the beginning of September 2013, when ½ of 5 l plants grown were replanted in V=11 l pots (s.c. “5→11 plants”). Then samples for analysis of dry matter partitioning and plant growth (De Groot et al., 2002) and thermodynamic calculations (Sun, 2002) were taken, while the remaining plants were transferred to full daylight (PARmax1500 μmol photon $m^{-2}s^{-1}$). After 7 days of light acclimation, 1/3 of plants were treated with 24-EBL (≈10^{-7} mol) solution, other 1/3 with PZR (propikonazole, an inhibitor of BRs synthesis (Hartwig et al., 2012); ≈10^{-6} mol) solution and 1/3 were control (K) plants. Treatment was repeated after 7 days. The parameters of Chla fluorescence (Maxwell and Johnson, 2000; Lichtenthaler and Miehe, 1997) were measured and calculated. After 2 weeks experiment was finished. During experiment, outside temperature was mainly suboptimal (t=14-19°C) for maize growth. In addition, we conducted the field trials, where we investigated the effect of foliar treatment with solutions of different concentrations (5.2 X 10^{-7}, 5.2 X 10^{-9}, 5.2 X 10^{-11}, 5.2 X 10^{-13} and 5.2 X 10^{-15} mol, and control) of 24-EBL, and two different solute concentration of PZR (≈10^{-6} mol and ≈10^{-7} mol), about a month after sowing (late April 2014) of maize (ZP434 and ZP341), as two weeks later. At the end August we sampled ears of corn, to assess the yield (per elementary plot (≈25 m^2) and per 1 ha) and its parameters, as well as the chemical composition of the grain (quantified by standard spectrophotometric and AAS methods).

Results and disscusion

Changes of allocation of dry mass between maize seedlings organs (plumule, radicle, the rest of the seed) indicate that the concentrations of 24-EBL from 5.2 X 10^{-12} M to 5.2 X 10^{-10} M most affected by the status of the radicle in both genotypes, as well as the status of plumule, but on different manner (Table 1). In contrast, the highest concentration of 24-EBL of 5.2 x 10^{-7} M has the most influence on the relative weight of the rest of the seeds in both maize genotypes (Table 1), so we can say that the allocation of dry weight from the rest of the seed to the plumula and the radikula of maize seedlings at that concentration 24- EBL is inhibited. In contrast to this uniform response of allocation of mass between organs and growth of maize seedlings, differential enthalpy, as a thermodynamic measure of the synthetic process, i.e. the thermodynamic potential of the system, are highly variable in both genotypes, depending on the temperature at which the organs of seedlings were dried (Table 1). As we know, enthalpy is defined as a thermodynamic potential of a system, i.e. as a measure of chemical potential of a system. In living systems reeling chemical reaction depends primarily on the water as a universal solvent. The most negative values of differential enthalpy testify to the fullest exothermal processes in certain organs of maize seedlings (Table 1), and various temperature at which the weight of seedling organs associated with various caps fractions of water in them (free (apoplastic) water, cytoplasmatic water, chemically bound water; Sun, 2002). According our data, changes of enthalpy of free, apoplastic water (dH105-60) affects the optimal 24-EBL impact on biochemical reactions at concentrations of 5.2 x 10^{-13} and 5.2 x 10^{-14} mol in radicle and plumule, while the least likely unwinding of biochemical reactions at concentrations of 24-EBL from 5.2 X 10^{-9} mol in ZP434 genotype (Table 1). In the case of changes of enthalpy of cytoplasmic (dH130-60) and chemically bonded (dH130-105) water of ZP434 maize plants, the concentration at which optimally convenient for 24-EBL influence on biochemical reactions are completely different (Table 1). In the case of changes of enthalpy of free water of plumule of ZP704 maize situation is oposite (data not shown).

Table 1. Average values (mean of 4 measures) of germination (%), relative fresh weight (RFW; g g^{-1}) and enthalpy (J mol^{-1} K^{-1}) of 25 maize (ZP434) seedlings parts (exposed at different concentrations of 24-EBL)

Treatment	Germination (%)	RFW (g g^{-1}) of radicules	RFW (g g^{-1}) of plumules	RFW (g g^{-1}) of rest of seeds	dH_{105-60}			$dH_{130-105}$			dH_{130-60}		
					Radicula	Plumula	Rest of seed	Radicula	Plumula	Rest of seed	Radicula	Plumula	Rest of seed
CONTROL	86	0.2685	0.2034	0.5280	-9,76657	-8,43709	**-8,33318**	-6,93085	-7,18778	-13,8514	-14,9590	-13,8416	-18,8396
5.2 X 10^{-7}	**41,5**	**0.13 50**	**0.08 92**	**0.7 642**	-8,15653	-7,57879	-6,93615	**-10,2 353**	-7,40911	-15,6683	**-15,8 970**	-13,1626	-18,8496
5.2 X 10^{-8}	75	0.1889	0.1074	0.6755	-8,68204	-7,90706	-7,5465	-7,46821	-6,82666	-13,6029	-14,2983	-13,0412	-17,8719
5.2 X 10^{-9}	89,5	0.2130	0.1202	0.6419	**-7,91389**	**-7,34894**	**-6,85003**	-9,32919	**-11,6316**	-12,7036	-14,9636	-16,1676	-16,495
5.2 X 10^{-10}	77	0.2508	0.1513	0.5979	-9,09034	-8,16918	-8,33831	-8,81979	-9,75917	-12,5936	-15,7365	-15,5451	-17,8819
5.2 X 10^{-11}	91,5	0.3306	0.1717	0.5275	-8,52971	-8,08468	-6,99159	-8,3960	-11,4415	**-16,9578**	-14,8580	**-16,7493**	**-19,8914**
5.2 X 10^{-12}	90	**0.33 55**	**0.24 44**	**0.4 202**	-10,3387	-8,77106	-8,09913	-6,42248	-6,08089	-10,1458	-15,1354	-13,3244	-15,7718
5.2 X 10^{-13}	92	0.2810	0.2007	0.5183	**-10,4219**	-8,75665	-8,22315	-5,46269	-7,60584	-9,96938	-14,4830	-14,4775	-15,7593
5.2 X 10^{-14}	87	0.3002	0.2275	0.4728	-10,3693	**-8,83346**	-8,30976	**-3,80279**	**-5,33537**	**-8,66468**	**-13,1604**	**-12,8154**	**-14,8462**
5.2 X 10^{-15}	**92,5**	0.3013	0.2278	0.4708	-10,4053	-8,67259	-7,74965	-5,10142	-7,1361	-10,6612	-14,1900	-14,0348	-15,8209

What is the cause of the observed differences in germination, the redistribution of mass and ability to perform biochemical reactions (differential enthalpy: dH) in various organs of the maize varieties, with the influence of the different concentrations of 24-EBL? Therefore, we analyzed the content of photosynthetic pigments in fresh, as well as some sugars (Table 2) and polyphenols (data not shown) in dried tissue of plant organs of maize seedlings. Low values of ratio's of photosynthetic pigments (Table 2) testify poor competence of the photosynthetic apparatus of plumule, which is not surprising for such an early stage of development of seedlings (Babani and Lichtenthaler, 1996). However, this means that most assimilates (i.e. and sugar), necessary for growth and development of plumule and radicle comes from the rest of the seed (Thomas and Rodriguez, 1994). We analyzed trehalose, sugar important in plant resistance to stress (Paul et al., 2008), followed by arabinose, an sugar moiety present in cellulose in cell wall of grass (Carpita, 1996), as well as in some important glycoproteins (Fincher et al., 1983), followed by glucose and fructose, sugars significant both

in primary metabolism, as well as necessary monomers of important plant polysaccharides (Duffus and Duffus, 1984), as well as sucrose, a main transport sugar in higher plants (Komor, 2000). Overall, the build-up of the aforementioned sugars is more pronounced at higher concentrations 24-EBL, and less at lower concentrations of mentioned phytohormone (Table 2). We think that the lower the concentration of 24-EBL accelerate biochemical processes and metabolic processes in plumule and radicle, which grow rapidly (germination!), but the plant does not suffer from osmotic stress (low concentration of trehalose!). A specificity of the metabolic processes in different organs of maize seedlings testifies by work of Waisi et al. (2015) that are water soluble polyphenols primarily related to radicle, and lypophylic polyphenols primarily related to plumule.

Table 2. Average (of 3 measurements) values of content of some biochemical parameters of different parts of a maize (Z. mays L.; ZP434) seedlings. RoS: rest of seed; P: plumula; R: radicula.

Treatments of 24-EBL	Chl a/b	Chla/carr (x+c)	Trehalose (µg/ 0.25 g of dry weight)			Arabinose (µg/ 0.25 g of dry weight)			Glucose (µg/ 0.25 g of dry weight)			Fruktose (µg/ 0.25 g of dry weight)			Sucrose (µg/ 0.25 g of dry weight)		
			RoS	P	R	RoS	P	R	RoS	P	R	RoS	P	R	RoS	P	R
CONTROL	0.541	**0.754**	73.90	43.88	62.40	**283.8**	**81.45**	55.20	**104626**	222.65	68.54	261.21	271.17	25438	130.17	649.21	842.12
5,2 X 10^{-7}	**0.498**	**0.447**	**39.66**	127.87	83.09	40.1	56.61	21.65	894.36	**238658**	14170	149.68	**269874**	43233	**495.42**	2103.84	**438553**
5,2 X 10^{-8}	-	-	70.16	**198.51**	74.16	134.16	**14.65**	26.63	874.85	469.59	**22119**	160.08	667.91	**54935**	225.71	135.42	368816
5,2 X 10^{-9}	0.542	0.504	84.79	115.72	32.24	4.37	21.63	**108.62**	430.48	184.65	72.40	117.11	252.8	11003	45.89	**88.65**	121003
5,2 X 10^{-10}	-	-	83.30	62.86	61.90	6.45	24.63	21.77	**275.12**	143.67	43.79	**77.59**	261.29	10760	76.20	100.03	278514
5,2 X 10^{-11}	**0.550**	0.509	207.71	**14.73**	31.53	7.03	40.87	15.92	563.56	113.19	39.70	79.45	286.91	24386	**38.77**	**3174.90**	299357
5,2 X 10^{-12}	-	-	226.90	20.23	22.28	13.9	32.78	14.87	560.78	**41.16**	49.86	116.66	112.24	11465	87.85	2599.47	133823
5,2 X 10^{-13}	0.538	0.557	180.52	36.12	30.63	**1.54**	67.54	23.64	544.51	251.86	12077	128.54	**101.49**	19949	98.13	2977.35	208933
5,2 X 10^{-14}	-	-	140.74	32.66	**3.01**	3.07	63.87	**0.88**	583.41	286.50	**2.67**	121.08	156.15	**24.84**	119.04	2622.72	**29.94**
5,2 X 10^{-15}	0.515	0.509	**333.75**	34.91	29.67	5.55	59.53	19.04	624.82	212.42	38.59	**162.65**	101.11	17846	178.84	2298.50	216961

In the next experiment, we monitored the effect of 24-EBL (≈10^{-7} mol), and propiconazole (≈10^{-6} mol) (an BRs biosynthesis inhibitor in plants; Hartwig et al., 2012) on growth,

allocation of dry weight in plant, thermodynamic changes during accumulation of dry weight, and parameters of photosynthesis in maize plants exposed to root manipulation during the trial (Table 3). Indeed, PZR treatment affected the root volume of “5 plants” (Table 3). However, if the change in free energy was considered in whole maize plant (ΔG_{105tot}) we note that this parameter is higher in “5 plants”, whereas PZR treatment (Table 3) increases change of free energy in the “5→11 plants”. So the “5 plants” have higher free energy values, indicating their higher susceptibility to the stress, but the reaction is modulated by the inhibition of BRs synthesis. It is notable that during the experiment the LMR was reduced and RMR rose of “5 plants”, regardless the way of BRs content manipulation in plants (Table 3). In “11 plants” or “5→11 plants”, we noted a slight LMR and SMR increase and RMR decrease; where treatment with PZR slightly emphasizes this trend (Table 3). In control and plants treated with 24-EBL, ΔG_{105} values of leaves and stems of “5 plants” and “5→11 plants” are lower than in “11 plants”, but in the case of root ΔG_{105} situation is reversed. In the plants treated with the PZR situation is inverted, meaning that the inhibition of BRs synthesis affects the change of free energy in different organs of maize plants (Table 3).

Table 3. Average (of 6 measurements) values of parameters of maize (ZP 505) plant growth and matter partitioning and thermodinamic changes during manipulation of root status and plant content of BRs

Treatments during trial	FW (g) leaves	DW (g) leaves	ΔG_{105} leaves (J mol^{-1} K^{-1})	LMR (g g^{-1})	FW (g) stem	DW (g) stem	ΔG_{105} stem (J mol^{-1} K^{-1})	SMR (g g^{-1})	FW (g) root	DW (g) root	ΔG_{105} root (J mol^{-1} K^{-1})	RMR (g g^{-1})	Vroot (ml)	TFW (g)	TDW (g)	ΔG_{105} tot (J mol^{-1} K^{-1})	$a_{W\,tot}$ (r. u.)
Start K 5	**4,94**	**0,46**	0,3526	0,561	**3,24**	**0,23**	0,1581	0,280	**0,68**	**0,13**	**0,6135**	0,159	-	**8,86**	**0,82**	0,3055	0,907
Start K 11	9,13	0,81	**0,5081**	0,587	6,45	0,35	**0,2713**	**0,254**	2,28	0,22	0,3490	0,159	-	17,86	1,38	0,2530	0,923
End K 5→11	29,28	2,83	0,3870	0,567	34,42	1,52	0,1684	0,305	4,50	0,64	0,5123	0,128	3,9	68,2	4,99	0,2390	0,927
End K 5	14,06	1,96	0,3601	0,492	15,01	1,16	0,1574	0,291	8,09	0,86	0,5163	0,216	5,5	37,16	3,98	0,3562	0,893
End K 11	36,18	3,19	0,5201	0,584	38,95	1,61	0,2518	0,295	5,09	0,66	0,3578	0,121	**3,8**	80,22	5,46	0,2218	0,932
End 24-EBL 5→11	28,74	3,11	**0,2981**	0,571	31,94	1,56	0,1383	0,286	5,15	0,78	0,5198	0,143	4,8	65,83	5,45	**0,2039**	0,937
End 24-EBL 5	13,38	2,04	0,3196	0,481	15,85	1,22	0,1420	0,288	**9,11**	**0,98**	0,4823	**0,231**	3,9	38,34	4,24	**0,3685**	**0,889**
End 24-EBL 11	**38,58**	3,49	0,4720	0,563	**46,01**	**1,98**	0,2529	0,319	4,79	0,73	0,3533	**0,118**	4,4	**89,38**	**6,20**	0,2261	0,931
End PZR 5→11	29,41	3,12	0,2902	0,590	31,2	1,53	**0,1327**	0,289	3,61	0,64	0,4366	0,121	4,3	64,22	5,29	0,2704	0,918
End PZR 5	12,80	1,91	0,3073	**0,457**	16,45	1,36	0,2315	**0,325**	8,66	0,91	0,6671	0,218	**6,8**	37,91	4,18	0,3675	0,890
End	30,	**3,5**	0,2	**0,6**	32,	1,	0,1	0,2	3,	0,	**0,3**	0,1	**3,**	67,	5,8	0,2	**0,9**

PZR 11	65	**5**	921	**08**	41	69	754	89	99	60	**190**	03	**8**	05	4	184	**33**

How applied treatments affect the photosynthesis of maize estimated by fluorescence of Chla? The “5 l plants” have high values of RC PS_2 activity indicators (Fv/Fm,Fv/F_0), except in PZR treated plants, while the values of the parameters are same in the “11 plants” (Table 4). It is similar situation in the case of parameters of photochemical efficiency (ΦPS_2, qP). All NPQ values (photoprotective processes indicator) were high, indicating that the plant fight stress (low temperature), regardless of treatment. Finally, two different indicators of overall photosynthesis (ETR, RFD_{730}) point that maize plant struggles with stress and the handling of brassinosteroids content had negative effect on “11 plants”. If we compare the above mentioned parameters we find that the thermodynamic parameter $\Delta G_{105\ tot}$ (J mol^{-1} K^{-1}) is significantly positively associated with the regression parameters of Chla fluorescence NPQ (R^2 = 0.2193) and RFD_{730} (R^2 = 0.2262) (data not shown). Furthermore, the dry root mass ratio (RMR; g g^{-1}) is significantly positively associated with regression thermodynamic parameters $\Delta G_{105\ root}$ (R^2 = 0.8416) and $\Delta G_{105\ tot}$ (R^2 = 0.3708), and Chla fluorescence parameters Φ PS_2 (R^2 = 0.1877), Fv/F_0 (R^2 = 0.1617), RFD_{730} (R^2 = 0.3741), NPQ (R^2 = 0.4091), and ETR (R^2 = 0.2063) (all results not shown). In contrast, the accumulation of total fresh weight (TFW; g) is in the negative regression related to thermodynamic parameters $\Delta G_{105\ root}$ (R^2 = 0.2425) and $\Delta G_{105\ tot}$ (R^2 = 0.3864), as well as the parameters of Chla fluorescence Φ PS_2 (R^2 = 0.4924), Fv/F_0 (R^2 = 0.0583), RFD_{730} (R^2 = 0.1807), and ETR (R^2 = 0.4472) (all data not shown). In a similar negative regression concerning there is a parameter accumulation of dry weight ln TDW (g) towards the thermodynamic parameters $\Delta G_{105\ root}$ (R^2 = 0.0658) and $\Delta G_{105\ tot}$ (R^2 = 0.0866) (Figure 4), and Chla fluorescence parameters Φ PS_2 (R^2 = 0.4910), Fv/F_0 (R^2 = 0,0079), RFD_{730} (R^2 = 0,0430), and ETR (R^2 = 0.4364) (data not shown).

Table 4. Average (of 4 measurements) values of parameters of fluorescence of Chla measured at youngest full developed leaves of same maize plants as in Table 3

Treatments during trial	Fv/Fm (r.u.)	Fv/F_0 (r.u.)	Φ PS_2 (r.u.)	qP (r.u.)	NPQ (r.u.)	ETR (µmol electrons m^{-2} s^{-1})	RFD_{730} (r.u.)
Start K 5→11	0,813	4,361	0,091	**0,278**	3,077	28,90	3,690
Start K 5	0,812	4,361	**0,206**	0,389	3,217	**49,06**	4,739
Start K 11	0,794	4,078	0,156	0,383	2,989	42,43	4,335
End K 5→11	0,786	3,756	0,100	0,305	3,144	21,55	3,925
End K 5	**0,839**	5,250	0,104	0,389	3,376	28,75	4,300
End K 11	0,793	3,836	0,107	0,389	2,944	33,56	3,711
End 24-EBL 5→11	0,836	5,117	0,180	**0,500**	3,876	45,53	5,228
End 24-EBL 5	0,837	**5,283**	0,151	0,333	**5,111**	39,35	**6,444**
End 24-EBL 11	0,792	3,822	0,088	0,389	3,126	22,77	3,788
End PZR 5→11	0,805	4,137	0,091	0,444	3,182	27,17	4,067
End PZR 5	**0,753**	**3,066**	0,153	0,472	3,194	38,55	4,183
End PZR 11	0,785	3,667	**0,081**	0,389	**2,799**	**18,47**	**3,485**

Table 5. Average (of 2 or 4 measurements) values of relative content (% against control) of different chemical and biochemical parameters in crude extract of ZP434 maize grain. Absolute values of control of different parameters: 1. starch: 74.60%; 2. total phenols: 260.05 µg/g; 3. moisture: 9.95%; 4. total proteins: 7.16%; 5. total oil: 3.45%; 6. Pphy: 3.22 mg/g; 7.

Pi: 0.36 mg/g; 8. GSH: 1053.63 nmol/g; 9. K: 3185.12 mg/g; 10. Ca: 36.38 mg/g; 11. Mg: 384.64 mg/g; 12. Fe: 5.08 µg/g; 13. Zn: 6.10 µg/g; 14: Si: 23.88 µg/g.

Relative content of different parameters (% against kontrol)	Treatments during trial							
	Control	5.2 X 10^{-7} of 24-EBL	5.2 X 10^{-9} of 24-EBL	5.2 X 10^{-11} of 24-EBL	5.2 X 10^{-13} of 24-EBL	5.2 X 10^{-15} of 24-EBL	10^{-6} PZR	10^{-7} PZR
starch	**100**	98.19	99.60	98.86	95.51	98.39	**95.17**	98.86
Total phenols	100	99.73	94.51	**148.63**	95.88	114.01	**92.03**	96.98
Moisture	100	**111.06**	**96.48**	104.52	108.04	108.04	110.05	105.02
Total proteins	**100**	108.72	101.19	105.58	**118.42**	102.51	115.42	107.47
Total oil	100	101.45	95.65	97.10	**105.80**	102.90	98.55	**94.20**
Pphy	100	100.73	95.62	**95.25**	99.03	102.31	103.16	**108.03**
Pi	100	**111.59**	100.29	96.01	107.98	98.10	97.44	**77.01**
GSH	100	122.21	**87.11**	110.69	**130.92**	107.73	104.02	117.43
K	100	99.33	95.76	98.25	96.19	**100.67**	97.99	**93.82**
Ca	100	79.90	122.53	145.37	478.45	89.92	**68.50**	**2755.82**
Mg	100	95.62	**78.81**	100.80	93.66	96.95	108.98	**112.02**
Fe	**100**	103.57	111.33	156.34	208.87	322.84	319.21	**384.17**
Zn	100	73.04	**49.26**	55.97	49.31	91.75	62.74	118.40
Si	100	118.65	88.89	80.20	88.01	99.16	**77.66**	127.72

In a field trial conducted in 2014 was not observed significant differences in yield (t/ha) between experimental treatments, except that it is somewhat (≈15%) was reduced in plants treated with 5.2 X 10^{-7} M 24-EBL (data not shown). Medium yield was 14.56 t/ha (18% grain moisture), ie. 13,977 t/ha (14% grain moisture), which is more than good. However, if we look at some yield components and some reserve matter content of grain (total protein, starch), the situation is somewhat different. In both hybrids, we see that only the treatment of 5.2 x 10^{-7} M 24-EBL reduces the number of rows in the piston (highly heritable trait), with from 14-16 to 12 (ZP341), ie. from 16 to 14 (ZP434), which is not observed in other treatment concentrations of 24-EBL (data not shown). We believe that this is due to a disorder of growth of maize plants treated with 5.2 x 10-7 M 24-EBL, which led to a change in these characteristic highly heritable trait. As for the characteristics of the number of kernels per row we see that in both maize hybrids that number in the control plants is about 38, among those treated with 5.2 x 10^{-7} M 24-EBL about 33, and in all other treated plants from 39 to 40 (data not shown), which means that treatment with 24-EBL has a positive effect on the characteristic. In addition, in hybrids ZP434 higher concentrations of 24-EBL increased content of biochemical parameters, but the implementation PZR as biosynthesis inhibitor of BRS, increases content of elements (Table 5). The situation is also similar in the hybrid ZP341 (data not shown). These facts are consistent with the literature data (Hola et al, 2010).

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THE IMPACT OF THE CUTTING OF RED CLOVER ON THE POLLINATORS VISITATION

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Abstract

Twenty honey bee colonies selected from the apiary of the Institute for forage crops in Kruševac (Serbia) were used for the experiments. The colonies were transported to and placed on the location where the red clover bred for seed was grown (Lukovo and Vrbnica). All colonies were inspected and their strength and food supplies determined. Bees were counted in five days in three different terms. All the bees that have left the hives (scouts) and those who were returning with a load of pollen (foragers) were counted. Counting lasted 3 minutes per colony per term of day. The activity of scouts and foragers differed depending on the red clover cut. The coefficient of variation for scouts was slightly higher in the second cut (10.7% in the second and 8.0% in the third cut). Similar results were obtained for foragers, where the coefficient of variation as the second cutting was 14.4% and 11.2% in the third cut. The largest number of scouts in the second cut was 465, and the third cut 373. Regarding foragers, in the second cut, the most counted foragers was 315, while the third cut, the largest number of foragers was 182. Percentage of honey bees in relation to other pollinators in the second cut was 75.0%, and at the third cut was 61.7%.

Keywords: *honey bee, red clover, pollination, variability*

Introduction

Red clover is one of the most important forage crops in Serbia and is a short-lived plant species which lives two to three years. It has a good tolerance to less favorable soils, which are not suitable for alfalfa, and also gives high yields of good quality forage (Mousset-Declos, 1993)

Red clover is almost entirely cross-pollinated forage crop (97- 98%). Self-sterility of this plant is caused by gamete incompatibility. Considering flower morphology, red clover is entomophile plant, and the most significant pollinators are honey bee (*Apis mellifera* L.), bumble bees (*Bombus* spp.) solitary bees and others, (Taylor and Smith, 1979). A lot of authors consider red clover a “bumblebee crop”, but there are those that give primary role to honey bee (Palmer-Jones, 1967; Jevtić et al., 2010). In red clover there is no naturally occurring side opening whereby bees can obtain nectar without tripping the flowers as may occur sometimes when honey-bees are collecting nectar from alfalfa flowers (Morrison, 1962).

The pollination of red cover depends on many factors, including the proximity of honey bee colonies to the red clover fields (Jevtić, 2010).

Rao and Stephen (2009), in their research, have found that in the isolation cages with bumble bees, red clover gave 661 kg ha^{-1} of seed, while in cages with honey bee 640 kg ha^{-1} of seed was obtained. Seeds yield of red clover can vary much and depends on the exploitation year and can reach a maximum of 700-1000 kg ha^{-1}. Seed yield in research of Lugić et al. (1996) depended on the seed norms and interrow distance.

The amount of nectar in the flower tube has the main influence on the honey bee visits to the red clover. Miladinović (1968) found that in the second cut 71% of flower tubes had more than 2 mm of nectar, and the 29% had nectar level less than 2 mm. The diploid cultivars of red clover (2n=14 chromosomes) are easier to pollinate than tetraploid cultivars (4n=28), since these have a deeper floral tubes, and so the seed production is considerably lower. Due to this, the increased interest in the honey bee for the pollination of red clover is quite justified.

The aim of this study was to determine whether the cutting of the red clover (second or third cut) had impact on the visitation by pollinators and thus the pollination of red clover.

Material and methods

Honey bees from the apiary of the Institute for forage crops in Kruševac city were used. Colonies were in LR hives, and 20 colonies were used for the research. The research was conducted in 2012. Before sampling, colonies in the apiary were inspected and colonies with similar strength and food supply were selected for further research. Selected 20 colonies were transported to the areas where red clover for seed production was grown. Selected sites were Lukovo in Boljevac district and Vrbnica in Aleksandrovac district. Red clover in Lukovo was in second cut, while the red clover in Vrbnica was in the third cut.

To determine the number of foragers (worker honey bees which bring pollen back to the colony) and scouts (worker bees which scout for nectar or pollen sources), bees were counted for 3 minutes, three times a day. The visitation to the flowers of red clover was determined by counting all pollinating insects that visited red clover flowers on 1 m^2. These countings were done on three separate lots (3 x 1 m2) in the same time. Countings lasted for 10 min and were repeated three times a day.

Statistical analysis was done using computer program Statistica 8.0 (StatSoft, 2008)

Results and discussion

There were differences in the number of counted honey bees depending on the cut of red clover. Total number of scouts in the second cut was 56101 and of foragers 26924, while in third cut total of 36954 scouts and 13855 foragers were counted. The largest number of scouts in the second cut was 465, and in the third cut 373. Regarding foragers, in the second cut, the most counted foragers was 315, while the third cut, the largest number of foragers was 182.

This is similar to the results of Rao and Anderson (2010) of their research of pollinator abundance during red clover bloom. According to their research, honey bee abundance dropped during late bloom and they speculated that this is due to honey bee preference for alternative pollen sources present in the area. Similar observations have been reported from other areas (Petersen et al 1960).

After the statistical analysis, it was determined that the activity of scouts and foragers differed depending on the red clover cut. The coefficient of variation for scouts was slightly higher in the second cut (10.7%) than in the third cut (8.0%). Similar results were obtained for foragers, where the coefficient of variation in the second cut was 14.4% while in the third it was 11.2%. In both cuts, honey bees were prevalent pollinators compared to solitary bees and bumblebees. Percentage of honey bees in relation to other pollinators in the second cut was 75.0%, and at the third cut was 61.7%.

Table 1. Average values for scouts and foragers counted in the second and third cut

	Scouts		Foragers	
Colony	Second cut	Third cut	Second cut	Third cut
1	234,6[a]	173,7[a]	115,2[a]	59,0 [ab]
2	199,7[ab]	139,2 [b]	100,6[ab]	50,2[b]
3	188,3[ab]	150,0 [ab]	97,1[ab]	62,9 [ab]
4	175,1[ab]	154,4 [ab]	75,9[ab]	49,7 [b]
5	199,9[ab]	163,6 [ab]	102,9[ab]	55,2 [ab]
6	196,1[ab]	162,2 [ab]	94,7[ab]	51,2[b]
7	206,9[ab]	155,2 [ab]	102,5[ab]	68,2[a]
8	177,0[ab]	159,4 [ab]	93,9[ab]	55,1 [ab]
9	156,1[b]	160,9 [ab]	75,2[ab]	52,2 [b]
10	180,1[ab]	149,3 [b]	73,4[ab]	61,4 [ab]
11	202,8[ab]	167,6 [ab]	88,9[ab]	52,7 [b]
12	211,7[ab]	163,4 [ab]	100,8[ab]	56,7 [ab]
13	188,7[ab]	162,7 [ab]	97,7[ab]	67,4[a]
14	181,6[ab]	132,2 [b]	87,3[ab]	56,8 [ab]
15	177,4[ab]	164,7 [ab]	84,5[ab]	53,3 [ab]
16	178,6[ab]	152,6[ab]	81,8ab	64,1 [ab]
17	175,1[ab]	155,3[ab]	81,8ab	65,4 [ab]
18	190,3[ab]	141,8[b]	97,9ab	61,2 [ab]
19	149,1[ab]	136,3[b]	67,5b	56,7 [ab]
20	163,6[ab]	134,7[b]	72,1ab	54,8 [ab]

a, b, ab – homogenous groups

There were three homogenous groups in both cuts for both scouts and forager honey bees. The colony number 1 have proved to be the one with the highest activity of both scouts and foragers in the second cut, while in the third cut it had the highest activity of scouts. The best activity of foragers in the third cut was found in the colonies number 7 and 13 (Table 1).

Conclusion

According on the results of this research, the pollination of red clover by honey bees depends on the cut. Higher activity of honey bees, both scouts and foragers, was determined in the second cut, while in the third cut it was diminished.

Honey bees were the dominant pollinators in both cuts, which might be a result of deliberate setting of the honey bee colonies on the red clover fields.

There were honey bee colonies which shoved better scout and forager activity, and those can be used for further selection of honey bees for more effective pollination of the red clover.

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THE EFFECT OF WATER STRESS ON SOYBEAN YIELD AND WATER USE EFFICIENCY IN A TEMPERATE CLIMATE

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Abstract

In Serbia, shortage of water limits soybean [*Glycine max* (L.) Merr.] production. Current irrigation practices aim at maximizing seed yield, but achieve lower return for the water consumed. Maximizing water use efficiency (WUE) may be more suitable in areas where water, not soil, is the most limiting factor. The aim of this study was to investigate the effect) of water stress on yield, WUE and irrigation water (IWUE) use efficiencies of soybean (*cv*. Nena) growing on a calcaric Chernozem in temperate environment. Field experiments were conducted at the experimental field of the Maize Research Institute “Zemun Polje”, in the vicinity of Belgrade (Serbia) during 2007 and 2008 growing seasons. The crop was subjected to three irrigation levels (rainfed and supply at 50 and 100% of field capacity, FC). Treatments were arranged in a randomized complete block design with four replicates. Rainfed (non-irrigated) and deficit irrigation treatment (50% of FC) significantly reduced seed yield. The full irrigated treatment (100% of FC) had the highest yield (3.06 t ha^{-1} average), while the rainfed treatment had the lowest yield (2.26 t ha^{-1} average), a 27% seed yield reduction. Deficit irrigation treatment produced 9% less seed yield then the full irrigated treatment. Both WUE and IWUE were significantly reduced as amount of irrigation water was increased. Seasonal crop evapotranspiration increased with increased amounts of irrigation water supplied. In conclusion, higher amounts of irrigation resulted in higher seed yield, whereas WUE and IWUE values decreased when irrigation amount increased.

Keywords: s*oybean, water stress, yield, water use efficiency, irrigation water use efficiency.*

Introduction

In the Vojvodina region, northern part of the Republic of Serbia, soybean is cultivated under both irrigated and nonirrigated conditions. In the period between 1993 and 2004 soybean was grown on 43,583–146,000 ha (Hrustić and Miladinović, 2008). According to Kazi et al. (2002), soybean crop seldom attains its full yield potential because limitations on physiological processes imposed by environmental stresses. The average yield was 2.1 t ha^{-1}, ranging from 1.2 to 2.8 t ha^{-1} and the yield levels depended primarily on the amount and distribution of precipitation within season. The introduction irrigation can potentially stabilize and increase yields, as well as increasing the use efficiency of water received both from precipitation and from irrigation. Under irrigated conditions, yields steadily exceed 3 t ha^{-1} and reach over 4 t ha^{-1} (Bošnjak and Dragović, 1998). In Vojvodina, irrigated yields were 22 % (average) higher, compared to those of nonirrigated soybean (Pejić et al., 2012). Cox and Jilliff (1986) reported that water stress reduced seed yield from 27 to 87% when the crop was subjected to a moisture deficit ranging from 18 to 70%. Kadhem et al. (1985) found that yield and other agronomic responses are sensitive to the water stresses during whole growing period. Burriro et al. (2002) studied the response of soybean to soil moisture stress in the environment oh the Pakistan. They found that the water use was affected by water stress. Considering paramount importance of proper use of irrigation for high yields, the present

studies were designed to investigate the effect of soil moisture stress on yield and water use efficiency in soybean crop in a temperate climate (Vojvodina).

Materials and methods

The field experimental (2007–2008) studies were carried out in Zemun Polje (44°52' lat. N; 20°20' long. E, 30 m a.s.l) at the experimental farm of the Maize Research Institute "Zemun Polje". The soil is a Calcareous Chernozem, silty clay loam – silty loam soil. It has field capacity (FC) of about 35% and wilting point (WP) at 16% by volume for the top 60 cm soil profile. Total plant available soil water is 173 mm m^{-1}. Mean bulk density varies from 1.17 to 1.41 g cm^{-3} (Tapanarova, 2011). The average annual rainfall was 638 mm (considering 20-year-long term period) and air temperature was 11.9°C. The climatic parameters during the crop growing seasons are summarized in Table 1.

The experiment was conducted using a randomized complete block design with four replications, and adapted to technical specifications of the sprinkling irrigation system. Each experimental plot size was 5 m^2. There was a 2.0 m space between each plot in order to minimize water movement between treatments. Soybean cultivar *Nena* was sown on 19 April in 2007 and 06 May in 2008. The sowing density amounted 445 000 plants ha^{-1}.

Common cropping practices were applied during vegetation. The following amounts of mineral fertilisers were used: 136 kg N ha^{-1}, 68 kg P_2O_5 ha^{-1} and 68 kg K_2O ha^{-1}. Weeds, pests and diseases were adequately controlled. The crop was harvested manually on 18 and 15 October in 2007 and 2008, respectively.

The crop was subjected to three irrigation levels (rainfed and supply at 50 and 100% of field capacity, were maintained on volumetric basis). Irrigation was applied when approximately 50% of the available soil moisture (the difference in soil water storage between FC and WP) was consumed in the effective root zone (to 0.60 m*)*. The irrigation rate and time were determined according to the soil water content at the effective root zone depth. Irrigation water was extracted from a deep well. Irrigation was started on 10 July 2007 and 04 July 2008.

Soybean evapotranspiration (ETa, mm) was estimated using the water balance method:

$$ETa = P + I + C_r - R_f - D_p \pm \Delta S \quad (1)$$

where P is the precipitation (mm), I is the irrigation water applied (mm), C_r is capillary rise to the root zone (mm), R_f is the surface runoff (mm), D_p is the drainage water (percolation) (mm), and ΔS represents the change in root zone water storage over a given time interval (mm).

Water use efficiency (WUE, kg ha^{-1} mm^{-1}) and irrigation water use efficiency (IWUE, kg ha^{-1} mm^{-1}) were estimated as Howell et al. (1995), (2, 3):

$$WUE = Y/ETa \quad (2)$$

$$IWUE = (Ym - Ya)/I \quad (3)$$

where Ym is the maximum harvested yield (under irrigation, non limiting conditions – 100% FC, kg ha^{-1}), Ya is the actual harvested yield (non-irrigated, kg ha^{-1}), ETa is the actual evapotranspiration (mm), I is the irrigation water amount (mm).

Grain yield in t ha^{-1} at 14% moisture was processed by the statistical method of the analysis of variances, while differences among individual treatments were analysed by the Fisher's test

(LSD) at the probability levels of 5% and 1%. The relationship between crop yield and total amount of water (precipitation + irrigation) was evaluated using regression analysis.

Results and discussion

Weather conditions, irrigation water applied and seasonal water consumption (ETa)

Total monthly precipitation and mean air temperature data during the soybean growing period are presented in Table 1.

Table 1 Mean air temperature and total monthly precipitation in 2007–2008 and between 1981 and 2002 at Zemun Polje.

Months	Temperature (^{o}C)			Precipitation (mm)		
	2007	2008	1981–2002	2007	2008	1981–2002
April	13	13	12	31	27	60
May	19	18	17	42	40	56
June	23	22	20	63	36	95
July	24	23	22	19	46	57
August	24	23	22	52	20	62
September	15	17	18	73	55	55
Total				279	225	384

In general, weather conditions were warmer and drier during the 2007 and 2008 growing seasons than to the long-term series. In both years, the average monthly temperature in the growing period was higher than the long-term temperatures, by 0.9^{o}C in 2007 and by 0.7^{o}C in 2008 (Table 1). During the summer months, there were particularly large deviation from the average temperature, and the soybean was then in the generative stages of growth when it was very sensitive to high temperatures and low relative humidity. High air temperatures, especially in the summer period 2007 (25.2^{o}C and 25.3^{o}C in third decade of July and August, respectively, Table 1) affected the amount of water used for evapotranspiration and irrigation frequency. The growing seasons from 2007 to 2008 were dry and very dry with 73 and 58% of the long-term mean precipitation (384.1 mm), respectively (Table 1). Because of the uneven seasonal distribution of precipitation irrigations had to be performed each year.

The irrigation rates depended on weather conditions during the investigation period. The amount of irrigation water applied in the growing season of 2007 was 135 mm and 50 mm for 100% FC and 50% FC, respectively. In 2008, irrigation rates were 235 mm and 95 mm for 100% FC and 50% FC, respectively.

Similar to irrigation water applied, seasonal water consumption (ETa) also depended on weather conditions during the investigation period. In the study period, ETa in non-irrigated conditions ranged from 226–308 mm and in the irrigation conditions in the range from 427–450 mm. The results observed in this study were in agreement with Pejić et al. (2012).

Yield

Table 2 provides information that the yield of soybean was significantly affected under irrigation treatments. The average yield at 100% FC (3.10 t ha^{-1}) was the highest, and significantly different compared to 50% FC (2.82 t ha^{-1}) and non-irrigated (rainfed) (2.26 t ha^{-1}). The average increases brought by supplemental irrigation were 0.84 and 0.56 t ha^{-1} or 37.2 and 24.8% under 100% FC and 50% FC conditions, respectively. Under the rainfed conditions, the yields of soybean depended primarily on the amounts and distribution of

rainfall during growing seasons. Our results are agrees with the results of Vučić and Bošnjak (1980) who had reported that the average increase of soybean yield by irrigation was 30%. Accordung to Pejić et al. (2012), the average yield increase of soybean due to irrigation practice was 0.82 t ha^{-1} on average, ranging from 2.5 t ha^{-1} in years with limited precipitation and higher than average seasonal temperatures (2000) to 0 t ha^{-1} in rainy years (1996, 1997, 1999). Carison et al. (1982) observed yield reduction due to water stress which varied from 20 to 50%.

Table 2 Yield (Y), water (WUE) and irrigation water (IWUE) use efficiency of soybean in grain production as affected by year and irrigation.

	Y (t ha^{-1})		Ave-rage	WUE (kg ha^{-1} mm^{-1})		Ave-rage	IWUE (kg ha^{-1} mm^{-1})		Ave-rage
	Years			Years			Years		
Variants	2007	2008		2007	2008		2007	2008	
Rainfed	2.88c†	1.63b	2.26	9.35ab	7.21b	8.28	–	–	–
50% FC	3.09b	2.55ab	2.82	9.48a	7.75a	8.62	4.20b	9.68a	6.94
100% FC	3.50a	2.69a	3.06	8.20b	5.98c	7.09	4.59a	4.51b	4.55
Cv‡	7.2	8.9		7.1	6.5		4.3	5.8	

†Means within columns not followed by the same letter are significantly different at the $P < 0.05$ level. ‡Coefficient of variation.

Contrary to our findings, Vučić and Bošnjak (1980), Bošnjak (1988) and Maksimovic et al. (2005) reported that irrigation variants with 70 and 80% of FC did not produce significantly different yields. Likewise, no significant differences could be found between the irrigation variants with 70 and 60% of FC (Maksimovic et al., 2005). Bennett and Albrecht (1984) reported that excessive soil water caused by unexpected precipitation following irrigation can affect soybean response to irrigation, resulting in lower yield. According to Mkasimovic et al. (2005), under conditions of high soil moisture, soybean develops large vegetative mass and it uses water excessively, so that the assimilation curve remains unaltered in spite of the increased water consumption.

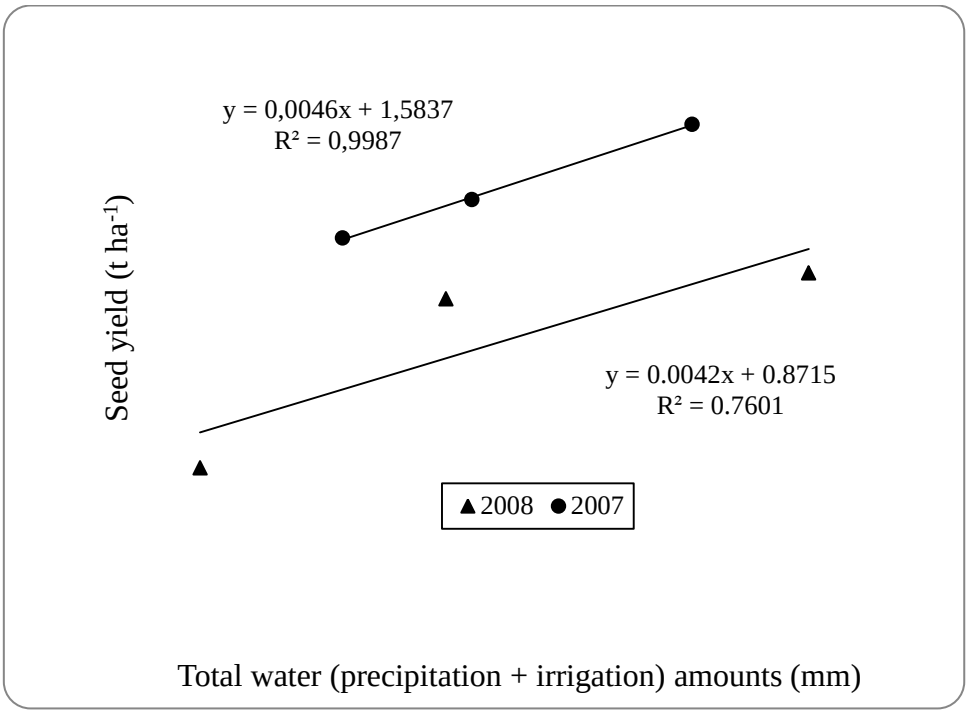
Water use efficiencies

Table 2 shows that the effects of deficit irrigation at vegetative period on the WUE and IWUE were significantly different. Irrigation treatments of 50 and 100% FC showed significant difference compared to rainfed (as a control). In this research, water use efficiency (WUE) of soybean ranged from 5.98 to 9.48 kg ha^{-1} mm^{-1} with an average value of 8.00 kg ha^{-1} mm^{-1}, while irrigation water use efficiency (IWUE) varied from 4.20 to 9.68 kg ha^{-1} mm^{-1} with an average value of 5.75 kg ha^{-1} mm^{-1} (Table 2). This agrees with the results of Pejić et al. (2012) in rainfed and irrigated soybean in Vojvodina.

In a 1-year experiment on the WUE of soybean growing on Ultisol soil, Bustomi Rosadi et al. (2007) found values of WUE of 0.037–0.187 kg m^{-3} (0.37–1.87 kg ha^{-1} mm^{-1}). Howell (2001) reported that irrigation can be an effective means to improve WUE through increasing crop yield especially in semiarid and arid environments. Even in subhumid and humid climate, irrigation is particularly effective in overcoming short-duration droughts. According to Pejić et al. (2012), irrigation by itself may not always produce the highest WUE possible.

The obtained IWUE values are in agreement with those given by Garcia y Garcia et al. (2010) who reported IWUE values of 0.55 kg m^{-3} and 1.14 kg m^{-3} in Georgia, USA. Demirtas et al. (2010) reported a lover values of IWUE (0.39-0.45 kg m^{-3}) in western Turkey as a consequence of very high seasonal evapotranspiration (684–771 mm) and comparative high

seed yield (3.57–4.00 t ha^{-1}). Scott et al. (1987) and Bhardwaj (1986) reported the average WUE soybean of 0.6 kg m^{-3} for USA and 0.63 kg m^{-3} for India, respectively.
The relation between total amount of water (precipitation + irrigation) and grain yield have been evaluated for each year (Graph 1). The relationship between total amount of water and grain yield was linear for each experimental year. Their relationship was significant at $P < 0.05$. A linear relationship between total amount of water and yield for soybean has been reported by other researchers (Garcia y Garcia et al., 2010).



Graph 1. Relationship between seed yield (t ha^{-1}) and total amount of water received during the growing season (precipitation + irrigation, mm).

Conclusion

Based on the results of the 2-year experiment on the effect of deficit irrigation on the yield and water use efficiency of soybean grown under temperate climate of the Vojvodina region, it was concluded that the supplemental irrigation practice brought highly significant yields compared with those obtained in the non-irrigated control. The average increase soybean yield due to irrigation ranged from 0.84 to 0.56 t ha^{-1} or 37.2–24.8%. IWUE decreased with increasing irrigation amount, with similar trends observed during both years; while WUE tended to increase in 2007 as irrigation amount increased, the opposite was in 2008. The finding of this study showed that variant 100% FC could be used for soybean grown in Vojvodina regions under no water scarcity. However, severe deficit in irrigation water amounts will cause significant declines in crop yield.

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COMPARATIVE ANALISYS OF PARAMETERS FERTILITY OF THE FORMS OF GRAPEVINE CULTIVAR TRAMINER IN THE SUBREGION OF NIŠ (SERBIA)

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Abstract

Researches included two Traminer cultivar forms: White Traminer and Traminer 11 Gm. The variety, as well as its clones and subvarieties, is described by many researchers, and all of them pointed out to its great variability in quantitative and qualitative traits. The study was done in the collection vineyard of the Center of Viticulture and Enology of Niš city (Serbia). The investigation lasted three years, and it included fertility, grape yield of the examined Traminer cultivar forms, resistance to *Botrytis cinerea* and wine quality. This paper also gives a detailed ampelographic description Traminer cultivar forms, according to International Organization of Vine and Wine (OIV) descriptors. The purpose of this paper is to determine growth, fertility, yield, and grape and wine quality of the examined Traminer cultivar forms in the viticultivar subregion of Niš. Average grape yield varied depending on conditions of the particular year. White Traminer recorded higher yield. Traminer 11 Gm wine has been awarded the average organoleptic grade of 18.17 points, which classifies it in the category of top-level white wines. Wine tasting grade awarded to White Traminer wine is 17.39 points, which classifies it as a quality wine. These researches contribute to the familiarization with the total variability of Traminer cultivar, as well as the importance of forms examined for the production of quality and top-level wines within climatic of the southern Serbia.

Key words: *Fertility, Ampelographic Description,White Traminer, Traminer11 GM, Form.*

Introduction

The occurrence of biotypes, forms within old grapevine cultivars is a well-known fact (Kaserer et al., 2003). Traminer is a very old and widespread cultivar with many biotypes characterized by diversity of genetic and phenotypic properties (Scienza et al., 1990). The first written data on Traminer cultivar was recorded in XV century (Goethe, 1887). The true origin of the cultivar is impossible to determine due to an extremely unstable genome. Traminer is much older cultivar than other grapevine cultivars, which is indicated by the high variation level (Imazzio et al., 2002). It is considered to originate from a Tyrolese place Tramin, Italy, wherefrom it has been cultivated in numerous European countries (Goethe, 1887). An important variability has been recorded within this cultivar. It is a heterogenous cultivar with numerous forms and clones. There have been attempts to proclaim certain forms for cultivars. White Traminer is grown in many European countries such as France, Austria, Switzerland, Germany (Santiago et al., 2007).

This Traminer form has been described by several authors (Galet, 1958; Foex, 1981; Ambrosi et al., 1994; Boidron et al., 1995).

White Traminer is by mistake frequently identified as a traditional Spanish cultivar Albarino Blanc (Santiago et al., 2007). Imazio et al. (2002) claim that Sauvignon Blanc is synonymous with White Traminer, which complies with Galet's (1990) ampelographic researches, according to which Sauvignon, i.e. White Traminer is one of Traminer cultivar forms. From a

population Traminer cultivar form Traminer 11 GM singled Helmut Becer (1979) at the Institute Geisenheim in Germany. In Serbian vineyards, Red Traminer dominates with numerous forms, which are more fertile than the cultivar population, yet at the same time of less intense fragrance which is especially valued in Traminer (Zirojević, 1974; Cindrić et al., 2000). The purpose of this paper is to apply standard ampelographic methods in order to record differences between the forms examined, to determine fertility, yield and quality of grapes and the wine produced under the ecological conditions of the subregion of Niš. This would contribute to the familiarization with biological properties, especially fertility of buds and shoots, as well as grape and wine quality that could be achieved in the ecological conditions of the subregion of Niš. These researches contribute to the familiarization with the total variability of Traminer cultivar, as well as the importance of forms examined for the production of quality and top-level wines within the climatic conditions of the southern Serbia.

Material and Methods

The investigation has been carried out during the period 2004-2006 in the collection vineyard of the Center for Viticulture and Enology at Niš. This grape varieties' collection is located in vinegrowing subregion of Niš, characterized by moderately continental climate with average annual air temperature of 11.8^{o}C and average vegetational air temperature of 18.1^{o}C. The absolute minimum of air temperature during the study was -18.2^{o}C. The average annual precipitation amount in the observed period was 750 mm, 422 mm of which fell during vegetation. Soil type was eutric cambisol. The vineyard was established in 1995, with planting distance of 3x1.2 m (2777 vines per ha), which was universal value for the all varieties at this collection vineyard. Bud load per vine was 20 buds or 6.6 buds per m^2. The trial was set in random complete block design with four replications, and the data were processed by analysis of variance. Ampelographic description has been done according to the descriptor list of OIV. Resistance to Botrytis cinerea was estimated by means of OIV descriptor, Code 459: 1 – 3 very low resistance, 5 – medium resistance, 7 – 9 high or very high resistance. Must quality, presented through the average content of sugar and total acid, was determined on representative samples during the vintage. Oechsle scale was used to measure sugar content, while titration with N/4 NaOH was applied in order to measure the total content of acid. Microvinification and chemical analysis of wine were carried out in the enological laboratory of the Viticulture and Wine Production Center of Niš. The quality of the wine produced was determined based on the results of the chemical analysis and organoleptic assessment made by the wine tasting commission of the Faculty of Agriculture, University of Belgrade. The all observed parameters were determined by standard ampelographic procedures.

Results and Discussion

Ampelographic description was carried out according to the OIV descriptors i.e. according to the Codes System which stipulates International Union for the Protection of New Varieties of Plants (UPOV) for a description of new genetic resources of grapevine.

Table 1. Ampelographic description of the Traminer forms (OIV, (1983)

Code	White Traminer	Traminer 11 GM
OIV 001	7	7
OIV 003	3	5
OIV 004	7	7
OIV 006	3	3
OIV 065	3	3
OIV 067	4	4
OIV 068	2-3	2
OIV 069	7	9
OIV 071	1	3

OIV 076	2	2
OIV 079	3	3
OIV 080	2	2
OIV 082	1	2
OIV 086	7	9
OIV 087	7	9
OIV 066	1	1
OIV 092	1	3
OIV 203	1	1
OIV 204	7	9
OIV 205	3	3
OIV 206	1	1
OIV 220	3	3
OIV 221	3	3
OIV 222	2	2
OIV 223	3	3
OIV 225	1	5
OIV 234	1	1
OIV 235	3	3
OIV 236	2	2
OIV 503	3	3
OIV 242	5	5
OIV 243	1	1
OIV 101	2	2
OIV 103	3	4
OIV 353	3	3

The examined Traminer forms did not show considerable differences based on the acquired data on ampelographic and botanical description of the tip of a young shoot, a leaf, a blossom, a flower, and a mature shoot (Table 1).

Table 2. The number of developed and fertile shoots

Forms	The number of developed shoots				The number of fertile shoots			
	2004	2005	2006	Average	2004	2005	2006	Average
White Traminer	18.42	16.50	18.33	17.75	16.00	13.83	16.08	15.31
Traminer 11 GM	18.67	16.42	18.00	17.69	16.33	12.25	14.08	14.22
Average	18.54	16.46	18.16	17.72	16.16	13.04	15.08	14.76
Lsd 0.05	Years 0.57		Forms 0.65		Years 1.18		Forms 1.36	

The number of developed and fertile shoots was not considerably varied between the examined forms of Traminer cultivar (Table 2).

Out of the total number of White Traminer shoots left at pruning, about 17.75 shoots, of which 15.31 fruit bearing ones, developed. As for Traminer 11 GM, 17.69 shoots, of which 14.22 fruit bearing ones, developed out of the 20 shoots left. In the examination years, meteorological conditions considerably influenced differentiation of fertile buds and the development of fertile shoots. In both forms, there were approximately as much developed and fertile shoots per vine as in the first and the third year, i.e. considerably more than in the second year of examination.

Table 3. Grape yield of the examined Traminer cultivar forms

Form	White Traminer				Traminer 11 GM				Lsd_{005}	
	2004	2005	2006	X	2004	2005	2006	X	Years	Forms
GYE (g)	145.6	107.9	148.6	133.99	134.6	90.5	111.5	112.24	17.32	20.00
GYDS(g)	156.1	131.1	160.5	149.27	144.2	110.6	123.9	126.28	18.95	21.88
GYFS(g)	178.5	155.6	180.4	171.53	163.2	148.4	155.9	155.88	15.62	18.04
GYV (g)	2912	2157	2969	2679.8	2692	1810	2231	2244.6	346.39	399.98
GYH (kg/ha)	8088	5992	8245	7441	7477	5027	6197	6233		
BW (g)	109.60	107.96	105.90	107.82	102.92	120.62	111.16	111.56	11.46	13.23

GYE – grape yield per bud, GYDS – grape yield per developed shoot, GYFS – grape yield per fertile shoot, GYV – grape yield per vine, GYH – grape yield per hectare, BW – bunch weight .

Yield parameters (yield per bud, grape yield per developed shoot, grape yield per fertile shoot, grape yield per vine, grape yield per hectare, bunch weight) varied significantly ($P<0.05$) between the examined forms and highly significantly between the examination years (Table 3). Average values of all yield parameters in White Traminer were significantly ($P<0.05$) higher than in Traminer 11 GM, excluding the grape yield per fertile shoot and the bunch weight. The highest yield per vine in White Traminer (2969 g) was in the third (2006) year, and the lowest (2157 g) in the second (2005) examination year. Traminer 11 GM had the highest yield per vine (2692 g) in the first (2004) year, and the lowest (1810 g) in the second (2005) year. The data obtained clearly indicated that the lowest yield in both forms was in the second year, namely much lower than in the other two years of examination. Grape yield per hectare varied in the same manner as grape yield per vine. White Traminer, with the average number of 25 bunches per vine, produced the average bunch weight of 107.82 g. On the other hand, Traminer 11 GM, with the average number of 20 bunches per vine recorded the bunch weight of 111.56 g. Cindrić et al. (2000) reported the average bunch weight of the forms examined under the conditions of Fruška Gora: Traminer 11 GM 130 g, and White Traminer 142 g. In our examinations, Traminer cultivar forms had smaller bunch weight than the one recorded by the above-mentioned authors. Differences in the bunch weight between the examined forms, and examination years as well, were not significant.
Bearing in mind that yield is mainly dependent upon the number of bunches and the average bunch weight, in our researches the yield was more dependent upon the number of bunches than on the average bunch weight. Taking into account biological features of Traminer forms, we can say that the yields there of during the research period were regular and low.

White Traminer showed a high level of resistance (7.0), while somewhat lower resistance was registered in Traminer 11 GM (6.3). The highest resistance level was registered in the first and third year (7.0), while the strongest *Botrytis cinerea* attack was registered in the second (2005) examination year (6.0).

Table 4. Resistance of Traminer forms to *Botrytis cinerea* (OIV Code 459)

Form	Examination year			
	2004	2005	2006	Average
White Traminer	7	7	7	7
Traminer 11 GM	7	5	7	6.3
Average	7.0	6.0	7.0	6.6

Data on the resistance level obtained in our researches are similar to the earlier reported data for Traminer cultivar.

Table 5. Content of sugar and total acid in must of Traminer cultivar forms

Form	Sugar content in must (%)				Total acid content (g/l)			
	2004	2005	2006	X	2004	2005	2006	X
White Traminer	23.59	21.18	22.41	22.39	7.48	7.88	7.50	7.62
Traminer 11 GM	22.11	19.54	20.64	20.76	8.18	8.60	8.80	8.52

A high content of sugar (22.39%) was registered in the must of the White Traminer form characterized by a high average yield (Table 5). Traminer 11 GM recorded less sugar content (20.76%). The lowest grape yield in the second year did not cause the increase of the sugar content in must. Traminer forms had a high content of acids in must, (8.07 g/l in average).

Table 6 data indicate that the alcohol content in wine is appropriately expressed, analogously to the sugar content. A higher alcohol content was obtained in White Traminer wine (13.33%). Content of the total extract varied from 17.25 g/l in White Traminer wine to 20.45 g/l in Traminer 11 GM wine. Content of the total acid varied within the limits of 6.5 g/l in White Traminer wine to 6.8 g/l in Traminer 11 GM wine. Traminer 11 GM wine has been awarded the average organoleptic grade of 18.17 points, which classifies it in the category of top-level white wines.

Table 6. Chemical composition and organoleptic assessment of wine

Form	White Traminer	Traminer 11 GM
Specific gravity	0.9893	0.9918
Alcohol (%)	13.33	12.23
Total extract (g/l)	17.25	20.05
Reducing substances (g/l)	1.0	1.05
Sugar-free extracts (g/l)	17.25	20.45
Titratable acidity (g/l)	6.5	6.8
Volatile acids (g/l)	0.35	0.34
Total SO_2 (mg/l)	69.5	83.0
Free SO_2 (mg/l)	10.0	13.5
Ash (g/l)	1.64	1.85
Phenolic substances (g/l)	0.16	0.18
Organoleptic assessment	17.39	18.17

Wine tasting grade awarded to White Traminer wine is 17.39 points, which classifies it as a quality wine.

Conclusion

Based on the three-year examination results of White Traminer and Traminer 11 GM forms under the conditions of the subregion of Niš, the following conclusions have been drawn:

According to the ampelographic and botanical description, between two examined Traminer grapevine forms (White Traminer and Traminer 11 GM) were not found big differences.

Yield of the examined grape forms depended more on the number of bunches than on the bunch weight. Average values of parameters of the White Traminer yield were significantly higher than those of the Traminer 11 GM yield, excluding the grape yield per fertile shoot and the bunch weight. The highest yield per vine in White Traminer (2969 g) was in the third (2006) year, and the lowest (2157 g) in the second (2005) examination year. Traminer 11 GM had the highest yield per vine (2692 g) in the first (2004) year, and the lowest (1810 g) in the second (2005) year. Grape yield per hectare varied in the same manner as grape yield per vine. White Traminer showed a high level of resistance to *Botrytis cinerea* (7.0), while somewhat lower resistance was registered in Traminer 11 GM (6.3).

Chemical composition of wines of the examined Traminer cultivar forms showed that they had high content of alcohol and a favourable content of the total acids. Organoleptic

assessment of the wine fluctuated between 17.39 points (White Traminer) and 18.17 (Traminer 11 GM), which could be considered highly satisfactory.

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THE EFFECT OF FOLIAR APPLICATION OF COBALT ON THE FORAGE YIELD OF RED CLOVER IN THE COMBINED FORAGE-SEED PRODUCTION

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Abstract

The crops of red clover (*Trifolium pratense* L.) are generally established on acid soils in which some of the trace elements are heavily accessible to the plants. In such conditions, the proper mineral nutrition can positively affect the yield of perennial legumes. The aim of the study was to analyze the effect of foliar treatment with cobalt on the forage yield of red clover on acid soil. The field experiment with varieties of red clover K-39, K-17, Una and Viola was set up in 2019, 2010 and 2011 in Čačak on alluvial soil type with pH(H_2O) 4.8. The experiment was designed using a randomized block design with four replications, with the plot size of 5x1 m. Sowing was carried out at 20 cm of inter-row distance and with seed rate of 18 kg ha^{-1}. The analyses were carried out on the first growth during the second year of cultivation, which in combined production forage-seed is mainly used for forage production. Foliar application of cobalt ($Co(NO_3)_2$ in concentration 0.033 g L^{-1}) was performed in the phase of intensive growth in the first growth in the second year of cultivation. The highest forage and hay yield in the control treatment was recorded in 2010, and the lowest in 2012, while on the variant with foliar application of cobalt there was no significant difference in the forage yield between 2011 and 2012. In the control treatment in all of the years, the variety Una had a significantly higher forage yield as compared to the varieties K-39 and Viola, while on the treatment with cobalt, the variety Una had a significantly higher forage yield only as compared to the variety Viola.

Key words: *red clover, cobalt, yield, forage, hay.*

Introduction

Given the economic and agro-tehnical importance of red clover *(Trifolium pratense* L.*)*, in the combined production of forage-seed, it is necessary to apply appropriate agro-tehnical practices to the potential for forage and seed yields a maximum realized. Proper mineral nutrition can have a positive effect on yield of perennial legumes, especially on acid soils (Dear and Lipsett, 1987). According to Taylor and Quesenberry (1996), acid soils are characterized by a high presence of easily accessible forms of aluminum, iron and manganese and reduced content of easily accessible phosphorus, calcium, and molybdenum. On acid soils, Al and H inhibited the growth of the root system, reducing its capacity for uptake of mineral nutrients and thereby reduces the resistance of plants to drought (Horst, 1991).

Growth and metabolism of plants to a large extent depend on the concentration of cobalt in the rhizosphere and soil (Palit and Sharma, 1994). Cobalt plant supply is essential for normal range of physiological reactions in the process of photosynthesis (Lipskaya, 1972), respiration (Palit et al., 1994; Aleshin et al., 1987), cell growth (increasing the amount of active form of auxin) (Lipskaya, 1970), which affects the rapid growth of plant organs (Ahmed and Evans, 1960; Mathur et al., 2006; Jayakumar et al., 2007; Jayakumar and Jallel, 2009). The positive effect of cobalt supply to plants is manifested through increased chlorophyll content (Lipskaya, 1972; Ozanne et al., 1963), increased thickness of palisade tissue, increased number and size of chloroplasts (Lipskaya, 1972). Cobalt significantly increases nitrogen

fixation by *Rhizobium*-all kinds, and thus the growth of legumes (Collins and Kinsela, 2011). Cobalt is a component of vitamin B_{12} that a component of enzymes and coenzymes involved in the process of nitrogen fixation in legume nodule (Bond and Hewitt, 1962; Kasimova et al., 1971; Palit and Sharma, 1994; Mathur et al., 2006). However, the high concentration of cobalt in plants is undesirable and it can have a detrimental effect on physiological and biochemical functions of the plant (El-Sheekh et al., 2003; Jayakumar and Jallel, 2009).
The aim of this study was to investigate the effect of foliar application of cobalt on acid soil on the forage and hay yield of red clover in the first cut in the second year of cultivation, which in combined production forage-seed is mainly used for forage production.

Material and Method

A field experiment was set up at a trial field of the Veterinary Extension Service in Čačak (43°54'39.06" N, 20°19'10.21" E, 246 m a.s.l.) in 2009, 2010 and 2011. The trial was established on alluvial soil (pH 4.8), which contained 3.18% organic matter, 0% $CaCO_3$, 22.1 mg P 100 g^{-1} soil and 30.0 mg K 100 g^{-1} soil. The preceding crop was natural meadow. In autumn, prior to seeding, 45 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5, and 45 kg ha^{-1} K_2O were incorporated into the soil by primary tillage.
The mean annual air temperature in 2010, 2011 and 2012 was 12.57ºC, 12.37 ºC, and 13.12 ºC respectively, and the sum of annual rainfall 669.4 mm, 374.5 mm and 463.5 mm respectively. The average annual air temperature for multy year period (1992-2002) was 11.97 ºC, and the average sum of annual rainfall 680.3 mm.
The experiment involving four red clover varieties and two treatments with cobalt (control, foliar Co) was laid out in a randomized block design with four replications and with a plot size of $5m^2$ (5x1m). Red clover varieties K-17, K-39 (Institute of Forage Crops Krusevac, Serbia), Una (Institute of Field and Vegetable Crops Novi Sad, Serbia) and Viola (a Polish variety) were planted at a row spacing of 20 cm and a seeding rate of 18 kg seed ha^{-1}. The crop was foliarly treated with Co (as $Co(NO_3)_2$), at a concentration of 0.033 g L^{-1} and a water rate of 1000 L ha^{-1}, applied once in the intensive growth phase during the first growth in the second year of cultivation. Mechanical weed control was performed twice in the first year of cultivation. No irrigation was employed.
The analyses were performed on the first cut in the second year of cultivation (2010, 2011, 2012) which in combined production forage-seed is mainly used for forage production. Mowing was done in the budding stage. Forage yield was determined by measuring the total weight of the plot immediately after cutting and recalculated to forage yield in t ha^{-1}. After drying at 65° C, the measured sample (500g) was used to calculate hay yield (t ha^{-1}). The obtained results were subjected to a mixed-design analysis of variance model (year as a random effect, varieties and foliar fertilization as fixed effects) using SPSS (SPSS 1995). Differences between means were tested by LSD test.

Results and Discussion

In the treatment without cobalt, the highest forage yield of the red clover varieties was recorded in 2010, significantly lower in 2011 and the lowest in 2012 ($P \leq 0.05$) (Table 1.). Considering that the crop was grown without irrigation, yield differences are mainly the result of differences in the amount of rainfall per year. At the variant with the foliar application of cobalt, the forage yield of the varieties in 2010 and 2011 did not differ significantly, while the yield in the both of the years was also higher, as compared to 2012 (interaction year/foliar fertilization). The extremely low amount of rainfall was recorded in 2012, during the growing season. At the treatment without application of cobalt, the varieties had a significantly higher hay yield in 2010 and 2011 as compared to 2012. In the variant with foliar application of cobalt, the largest hay yield was recorded in 2011, significantly lower in 2010, and the lowest

in 2012 ($P \leq 0.05$) (interaction year/foliar fertilization). Hanson (1991) reported that the forage yield of perrenial legumes reduces if soil moisture is inadequate. According to Bošnjak (1993) the average increase of forage yield of alfalfa in dry years with the implementation of irrigation amounted to 56-61%, whereby the effect of irrigation at the start of vegetation was somewhat more modest. According to Duncan and Woodmansee (1975), the correlation between precipitation and forage yield at grasses and legumes was significant during the phase of intensive growth, especially if in this stage is expressed the lack of soil moisture. Bošnjak et al. (1995) found at alfalfa the existence of full corellation ($r=0.99$) between the forage yield and the annual precipitation.

Considering that in the treatment without foliar application of cobalt was recorded a significantly higher forage yield of the varieties in 2010 in relation to 2011 and that on the treatment with foliar application of cobalt, the forage yield did not significantly differ in these two years, the foliar treatment with cobalt achieved a positive impact on the forage yield of red clover varieties in 2011. The hay yield is in accordance with forage yield; at the treatment without cobalt the varieties did not differ in 2010 and 2011, while at the variant with cobalt, a significantly higher yield of hay was recorded in 2011 as compared to 2010. The results among others are the consequence of a somewhat higher water content in forage in 2010 as compared to 2011. According Vrany et al. (1978), Powrie (1964), Ozanne et al. (1963), foliar applications of cobalt resulted in a significant increase in the number of nodules on the root system, the amount of fixed nitrogen, which resulted in more intensive growth of red clover. Reddy and Raj (1975) also indicate that foliar application of cobalt in soybean affects better nodulation and increase in seed yield. According to Jayakumar et al. (2007), low concentrations of cobalt (less than 50 mg kg^{-1} soil) have a positive impact on growth of soybean plants, and adsorption of nutrients from the soil. Cobalt in the form of fertilizer increases the yield of peanuts (Joshi et al., 1987) and pea (Danilova et al., 1969) as a result of increasing the number of nodules per plant. Accordingly, Dilworth et al. (1979) stated that in absence of cobalt, the weight of nodules per lupine plant reduced to 50-80%. Application of cobalt in concentrations of less than 50 mg kg^{-1} soil in beans influenced significantly increase the number of nodules per plant, number of effective nodules per plant, weight of effective nodules per plant, dry matter accumulation in plants, increasing the number of pods per plant (Mathur et al., 2006) and yield per hectare (Pattanayak et al., 2000; Mathur et al., 2006). It should be emphesized that any method of application of cobalt (foliar or in soil) may increase its concentration in the feed. Reith and Burridge (1983) recorded the increase of the content of cobalt in forage of several species of clover and ryegrass, by the application of 0.5 kg ha^{-1} of cobalt in form of cobalt sulphate, especially on peat soils.

Irrespective of the year, on the treatment with cobalt, the varieties also behaved differently in terms of forage yield, in relation to the treatment without cobalt. In the variant with foliar application of cobalt, in all of the years, the variety Una had a significantly higher yield compared to varieties K-39 and Viola, while on the treatment with cobalt the variety Una had a significantly higher forage yield only as compared to the variety Viola (interaction cultivar/foliar fertilization). According to Radović et al. (2004), red clover cultivars showed better results in agroecological conditions in which they were selected. According to the same authors, native varieties of red clover have an advantage over foreign varieties, as a result of better adaptability to existing environmental conditions.

Table 1. Forage yield - FY (kg ha^{-1}) and hay yield - HY (kg ha^{-1}) cultivars of red clover in the first cut in the second year of cultivation depending on the foliar treatment with cobalt: Ø – control, without cobalt; Co – cobalt (interaction year/foliar treatment; cultivar/foliar treatment and year/cultivar/foliar treatment).

		FY	HY
Ø	2010	48.81 a	7.19 b
	2011	39.85 c	7.53 ab
	2012	19.14 d	4.07 c
Co	2010	46.00 ab	6.87 b
	2011	42.41 bc	8.34 a
	2012	19.89 d	4.46 c
Ø	K-39	33.65 bc	5.94
	K-17	38.27 ab	6.40
	Una	39.58 a	7.18
	Viola	32.23 c	5.53
Co	K-39	34.63 abc	6.26
	K-17	36.60 abc	6.81
	Una	39.40 a	7.27
	Viola	33.77 bc	5.90
Foliar treatment		ns	ns
Foliar treatment x Year		*	*
Foliar treatment x Cultivar		*	ns
Foliar treatment x Cultivar x Year		ns	ns

The values denoted with different small letters within columns are significantly different at ($P \leq 0.05$) in accordance with the LSD test; * - F test significant at $p \leq 0.05$; ns - F test non-significant.

Conclusion

On the treatment without cobalt, the varieties realize the highest forage yield in 2010 with the highest rainfall, and the lowest in 2012 when the rainfall was the lowest. However, on the variant with foliar application of cobalt, forage yield of the varieties did not significantly differ between 2010 and 2011, and in both of the years the forage yield was higher as compared to 2012. The achieved results indicate that foliar treatment with cobalt achieved a positive impact on forage yield of the varieties of red clover in 2011, because on the variant with foliar application of cobalt the forage yield of the red clover cultivars did not differ significantly between 2010 and 2011. The hay yield results are consistent with the results of forage yield, because on the treatment without cobalt the varieties did not differ significantly in 2010 and 2011, while on the variant with cobalt a significantly higher hay yield was recorded in 2011 as compared to 2010. On the variant with foliar application of cobalt in all of the years, the variety Una had a significantly higher forage in relation to the varieties K-39 and Viola, while on the treatment with cobalt the variety Una had a significantly higher forage yield only in relation to the variety Viola.

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EFFECT OF FERTILIZATION AND MULCHING ON YIELD OF PEPPER

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Abstract

In a field trial set up on the research field of Agricultural station in Sombor, the effect of different fertilization systems and mulching on yield of pepper (varieties) and mineral nitrogen (N) dynamics in soil were studied. Trial was conducted during 2012 and 2013 cropping seasons. Pepper was grown with and without plastic foil as a ground cover, and on both mulching systems it was applied 240 kg N ha^{-1} in total, through different fertilization systems. Four organic fertilizers were applied: mature cattle manure (MCM); composted pig manure (CPM); vermicompost (V) and organic fertilizer *Fertor* (F). Each organic fertilizer was applied in two different amounts which contained 120 and 240 kg N ha^{-1}. On the same plots, mineral N fertilizers were added to fulfill target value for N application (240 kg N ha^{-1}). The monitoring of NO_3-N in soil during the pepper growing season has shown that the mineralization of organic fertilizers was most intense in the first half of vegetation, while in the second, during the yield formation, concentration of mineral nitrogen in soil was significantly reduced. Concentration of mineral N in soil under plastic mulch was significantly higher compared to plots without mulch. Yield of pepper in first and second term of harvest were positively correlated with concentration of mineral N in soil.

Key words: *soil, NO_3-N, fertilization systems, pepper, yield.*

Introduction

Vegetable cultures produce high yields during a short vegetation period so they have greater demands for nutrients, primarily nitrogen, which is an essential factor affecting yield.

In order to achieve high and stable good-quality yields in a short vegetation period of vegetable crops, not only mineral but also organic fertilizers need to be applied. Mineral fertilizers are fast-acting sources of nutrients. Organic fertilizers have a twofold effect on crops as they not only provide nutrients for the crops but they also positively affect the physical and biological properties of the soil. Organic fertilizers contain higher proportions of nutrients in an organic form, which is only after mineralization transformed into plant-available forms. It is therefore necessary to access the mineralization potential of organic matter in the fertilizer by following its nitrate dynamics so that the choice of an appropriate fertilizer and the time of its application can be coordinated with the demands of the crops (Čabilovski, 2009).

Nitrate dynamics in the soil is affected by numerous factors, the most important of which are temperature and humidity. Soil humidity directly affects the mineralization process and thus the mineral nitrogen content in the soil.

Low or excessive humidity can be stressful for microorganisms in the soil and it can inhibit their growth (Stanford and Epstein, 1974; Myers *et al*., 1982; Bogdanović *et al*., 2007; Ubavić *et al*., 2005), while alternating high and low levels of humidity increase the amount of fast-mineralizing material.

Temperature directly affects the decomposition of organic matter, while the microorganisms that are involved in the process have different temperature oscillations. Vegetable production in an open field occurs in the period of constant increase of soil temperature (10-15-25-30ºC) and, as intensive vegetable production is not possible without irrigation, this means that there

are favourable conditions for the mineralization of applied organic fertilizers and organic matter in the soil.
The rate of organic fertilizer mineralization is determined by the type of fertilizers, the degree of decomposition of organic matter, temperature, humidity and microbiological activity (Pansu and Truries, 2003). Due to intensive mineralization, an excessive application of organic fertilizers can lead to an increased accumulation of NO_3-N in the soil, harmful accumulation in the plants and pollution of the environment.
The objective of this research is to assess the effect of different systems of fertilizing pepper grown under polyethilene (PE) foil and without PE foil on NO_3-N dynamics in the soil and their effect on the yield.

Material and Methods

The dynamics of NO_3-N in pepper production was observed in field experiments set up using a split plot design with and without PE foil. The experiments are two-factorial, where factor A is the fertilization systems and B is the use of PE foil as mulch Pepper was grown with and without plastic foil as a ground cover, and on both mulching systems it was applied 240 kg N ha^{-1} in total, through different fertilization systems (Table 1). Each organic fertilizer was applied in two different amounts which contained 120 and 240 kg N ha^{-1}. On the same plots, mineral N fertilizers were added to fulfill target value for N application (240 kg N ha^{-1}).

Table 1. Treatments of fertilization and mulching

Source of nitrogen*	**Treatments of nitrogen fertilization**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Percent of nitrogen added by fertilization (100% = 240 kg N ha^{-1})									
NPK	0	100	50	0	50	0	50	0	50	0
MCM	0	0	50	100	0	0	0	0	0	0
V	0	0	0	0	50	100	0	0	0	0
F	0	0	0	0	0	0	50	100	0	0
CPM	0	0	0	0	0	0	0	0	50	100
	* NPK= mineral fertilizer; MCM = mature cattle manure; V = vermicompost; F = composted organic manure FERTOR; CPM = composted pig manure									
	Mulch treatments (MT)									
MT+	Use of black polyethilene foil (BPF) as mulch for 1-10 N treatments									
MT-	Without use of black polyethilene foil (BPF) for 1-10 N treatments									

Table 2. Agrochemical properties of soil in exspperiments before pepper planting

Year	Depth	pH		Percent			mg $100g^{-1}$		mg NO_3-N kg^{-1}	
		H_2O	KCl	$CaCO_3$	Humus	Total N	P_2O_5	K_2O	before planting	after planting
2012	0-30	7.60	7.0	4.59	3.12	0.16	21.9	22.1	36.0	27.0
	30-60	7.80	7.0	5.42	2.96	0.15	14.3	21.0	41.6	38.0
2013	0-30	8.01	6.85	3.82	2.48	0.12	17.8	21.3	46.4	30.0
	30-60	8.11	6.98	13.58	1.99	0.10	6.7	16.5	22.1	23.0

For the analysis of soil fertility standard methods were used. The first and the second harvests were performed during optimum technological maturity. The research results were processed by variance analysis for a two-factorial experiment.

Results and Discussion

NO_3-N dynamics in the soil under pepper with and without foil, dependent on applied fertilization systems, and fertilization treatments in 2012 and 2013 is given in Figure 1 and Figure 2. In both years a higher amount of NO_3-N was detected at all sampling times, with all systems and fertilization treatments in pepper under foil than in pepper without foil.

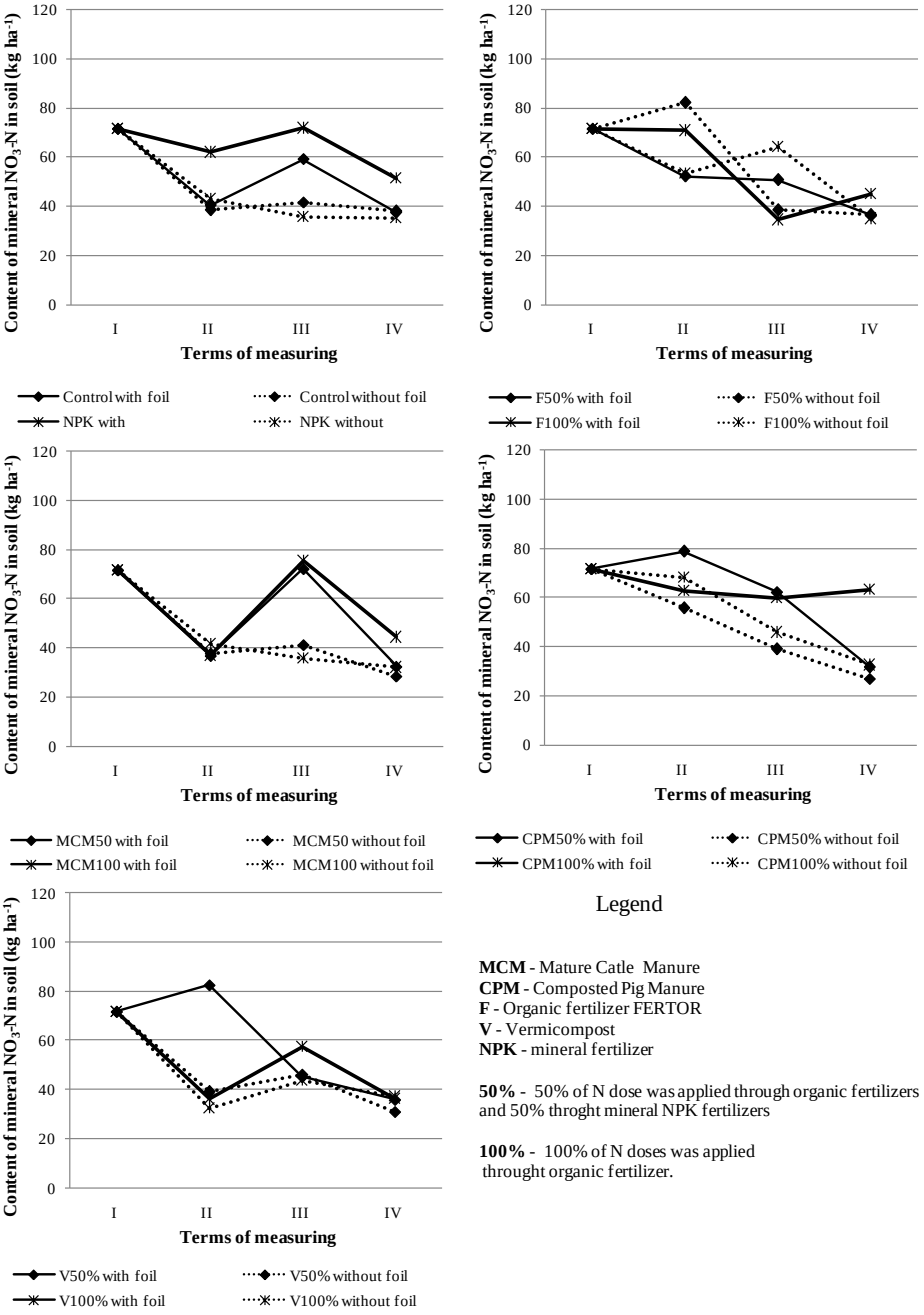

Figure 1. NO_3-N dynamics in the soil under pepper with and without foil, dependent on applied fertilization systems, and fertilization treatments in 2012.

Significantly higher amounts of NO_3-N were found in the soil under foil than in the soil without foil because of better mineralization conditions. The basic prerequisite for a better mineralization of organic fertilizers is a 3-4^oC higher temperature and a 1.5%-2.5% higher soil humidity in the experiment under foil when compared to the experiment without foil. The exception is the sampling made during intensive growth in 2012, in which the sample taken from the plot with pepper without foil had a significantly higher amount of NO_3-N with all fertilization treatments.

The highest amount of NO_3-N in the soil under foil was recorded in the experiment in 2012 with 100%. Fertor fertilization systems, and on the plots without foil with the application of N from vermicompost.

In the first harvest, the highest value of NO_3-N in the soil in 2012 was recorded with the treatment N from 100% MCM, and in the second harvest with the treatment N from 100% CPM under foil.

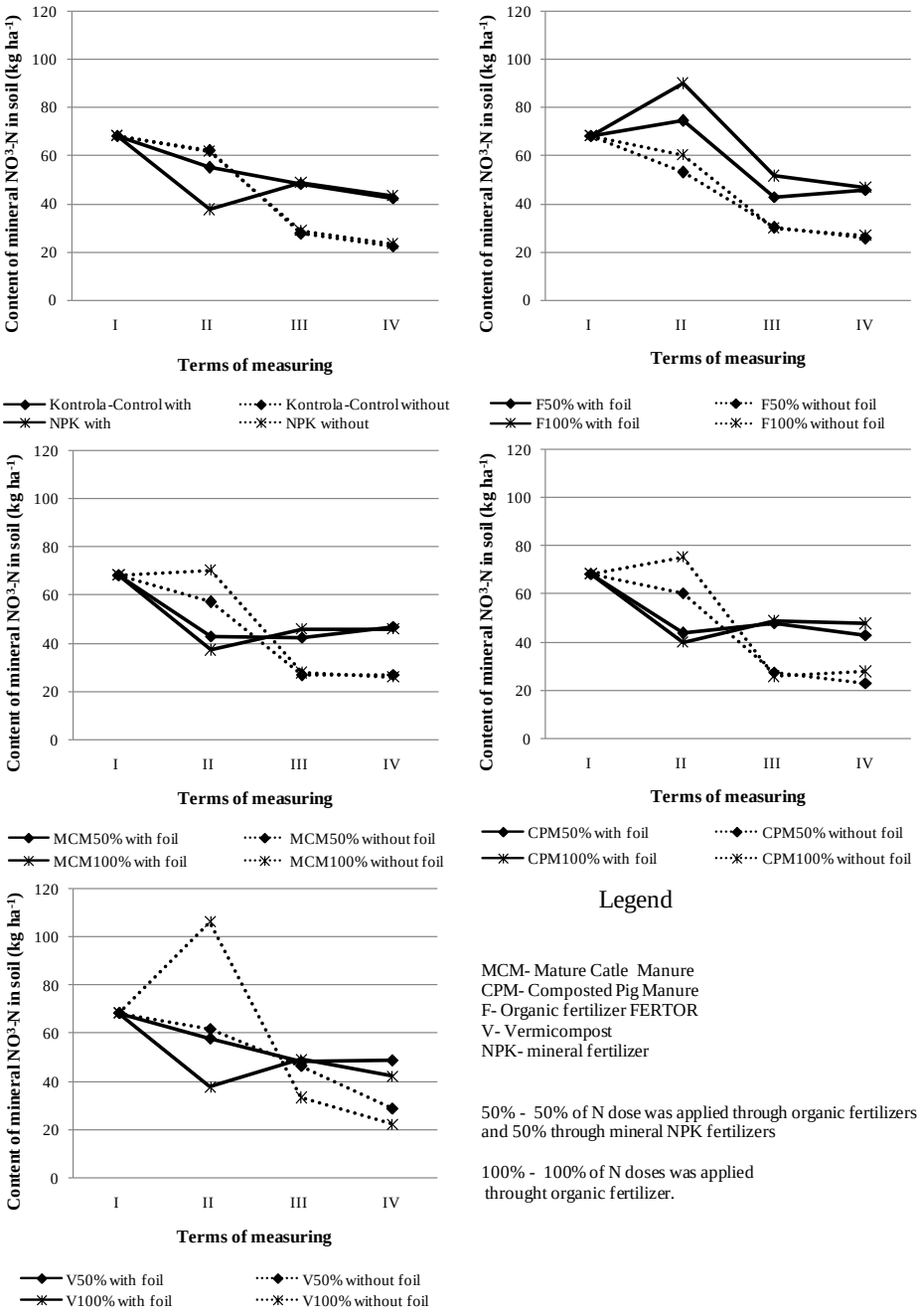

Figure 2. NO_3-N dynamics in the soil under pepper with and without foil, dependent on applied fertilization systems, and fertilization treatments in 2013.

The recorded values of NO_3-N under pepper with and without foil at different sampling times indicate that organic fertilizer N mineralization was more intensive in the first period of vegetation, during intensive growth, as compared to the second period of vegetation, when lowest values were recorded at the end of the second harvest.

The recorded amounts of NO_3-N at different sampling times represent a state of equilibrium between the mineralization of applied organic fertilizers and soil organic matter on one hand, and the immobilization of mineral nitrogen by pepper crops and microorganisms on the other (Bogdanović and Čabilovski, 2007; Bogdanović and Ubavić, 2008; Čabilovski *et al*. 2010).
NO_3-N dynamics in soil under pepper in the experiments with and without foil correlates positively with obtained yields in both years. The yields in the experiments with foil were significantly higher with all fertilization systems and treatments than those produced in the experiments without foil Figure 3 and Figure 4. Higher yields in the experiment without foil are directly dependent of the amount of NO_3-N measured in the soil, which area result of better conditions for organic fertilizer mineralization (humidity and temperature). Of all the applied organic fertilizers, the highest yields with and without foil were obtained with fertor (F) treatment, followed by composted pig manure (CPM), vermicompost (V), mature cattle manure (MCM), and the lowest with mineral fertilizers (NPK) (Figure 3 and 4).
The stronger effect that fertilizing with fertor had on pepper yields when compared to the other applied organic fertilizers stems from the production technology of this fertilizer and its content. Fertor is a pelleted organic poultry manure to which other organic plant materials are added and it contains all biogenic elements.
Furthermore, with CPM treatments higher yields were obtained with and without foil than with V, MCM and NPK treatments, which can be explained with significantly higher solubility and the presence of biogenic elements, primarily nitrogen from CPM, when it is compared to other fertilization systems**.** Research by Denić (2010) on maize and Bogdanović *et al*. (1995) on wheat shows that NO_3-N from liquid pig manure in the year of application had an effect on yield, identical to that of mineral fertilizers.

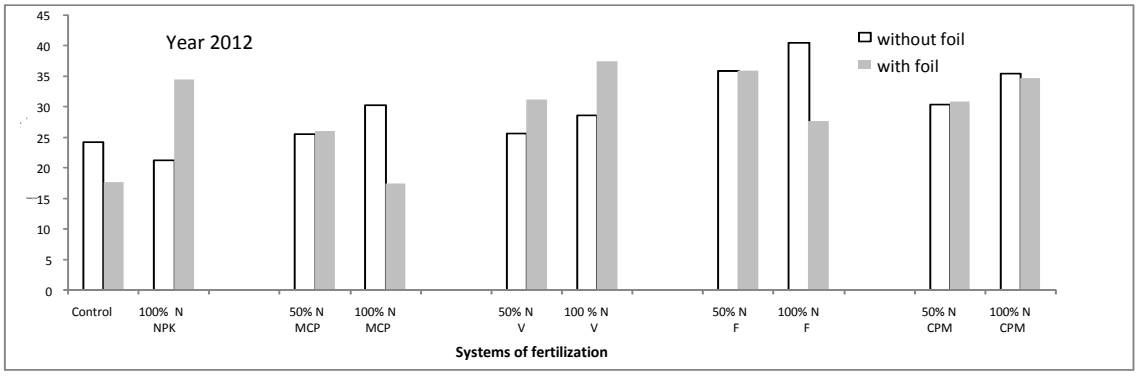

Figure 3. Pepper yield as dependent on fertilization systems and mulching in 2012.

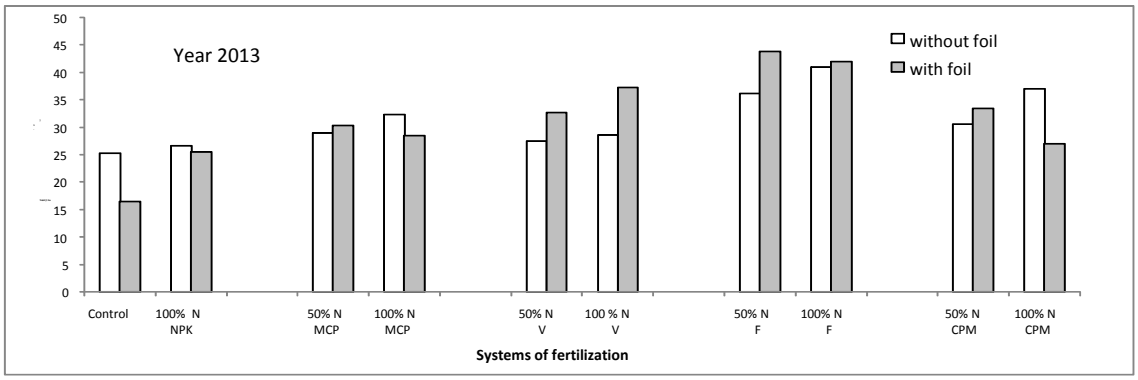

Figure 4. Pepper yield as dependent on fertilization systems and mulching in 2013.

Conclusion

Based on the observed dynamics of NO_3-N in the soil under pepper with and without foil, depending on the fertilization method and its effect on yield, the following conclusions can be drawn:

Mineralization of NO_3-N from organic fertilizers was more intensive in the first period of vegetation, during intensive growth, and considerably slower in the second period of vegetation at the times of the first and second harvest.

The highest amount of NO_3-N was recorded in the soil under pepper with foil in 2012, at the first harvest with N from 100% MCM treatment, and at the second harvest with N from 100% CPM treatment. In both years higher amounts of NO_3-N were found with foil than without it, at all sampling times, with all fertilization systems and treatments. Pepper yields under foil with all fertilization systems and treatments are significantly higher than those without foil.

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HARVEST INDEX AND COMPONENTS OF YIELD IN WINTER WHEAT CULTIVARS (Triticum aestivum L.)

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Abstract

Variability of grain harvest index (GHI) and some components of yield (plant height, thousand grain weight) were studied in 20 winter wheat cultivars which selected in in different selection centers. Wheat cultivars were grown in the experimental field in 5 replication. Samples of 100 wheat plants (20 plants in 5 replications) were analyzed in full maturity stage during two seasons. Harvest index was computed as a ratio of grain yield and a total above ground part of the plant. The values of GHI varies depending from cultivars and environment. The obtained values for all analyzed traits were significantly different among wheat cultivars. The highest harvest index in first year had cultivar Lasta (41.3%) and the lowest had Zastava cultivar (35.1%). In second years, the highest HI had cultivar Pobeda (42.6%) and the least GHI had cultivar Ljubičevka (33.2%). Wheat cultivar Zastava had the highest height in both year of investigation, 98.32cm in the first year and 78.84cm in the second year of analysis. The lowest plant height had Gruža culivar in both year, 70.56cm in the first year and 57.10cm in the second year. Thousand grain weight (TGW) in the first year variate from 38.33g in Gružanka cultivar to 51.12g in Zadruga cultivar. In second year the highest value of TGW had Zadruga (52.85g) and the least value 38.84g had the Ljubičevka cultivar.

Key words: *wheat, cultivar, grain harvest index (GHI), stem height, thousand grain weight (TGW)*

Introduction

In the wheat breeding program breeders have aim to improve all characteristic, what is not simple because of different genetic control of traits, and very complex relationship among the traits. For the successful breeding process is necessary use the knowledge of characteristics of genotypes as well interaction of genotypes and environments. The breeding of wheat is directed to creation the cultivar for high grain yield or for any other desirable traits need to express genetic potential in different environment with low value of variance in different environmental factors of growing (Knežević, 2014). The genetic yield potential of a wheat cultivar may be dependent on favorable conditions and good agronomy for its expression. The traits as height of plants, length of spike, number of spikelets per spike are in positive correlation with grain yield (Zečević et. al, 2013). The harvest index (HI) of wheat plants can use as reliable parameters in wheat breeding which varies from 5-17% in the wild diploids to 51% in modern cultivars. GHI has increased over time due to breeding for higher yield which achieved 0.015 for Australian cultivars, and 0.02 percent per year for United Kingdom cultivars (Turner, 1997). The harvest index represents the ratio between economical yield and biological yield i.e. ratio between grain yield and yield of r straw + grain. In mostly modern cultivars, the proportion of total mass to grain yield is more than 2:1 which indicates excessive consumption of nutrients for straw formation instead of grain. The high HI value can achieve through decreasing vegetative part of wheat and increase grain filling by application of mineral nutrition and increasing efficiency of utilization and translocation

nutrient (Gorjanović et al., 2011) to grain especially at the grain filling phase. Numerous environmental factors have influence to the value of HI as well other yield components. The low or high temperature can cause damages on seed growth and at flowering phase and can influence to significant variation in harvest index. Frost after anthesis can cause reduction in HI and total grain yield as well quality (Allen et al., 2001). The variability of growing wheat plant influenced by N status in soil in conjunction with available water and vigorous growth can contribute to reduction of HI of wheat (Duggan et al., 2005). Diseases and pests have negative influence to grain filling and size what implicate to forming of grain yield. Investigation of McKirdy et al. (2002) showed that yellow mosaic virus have influence on expression shriveling of grain in wheat.

The aim of this work is investigation of variability values of harvest index, height of plants, thousand grain mass in genetically divergent wheat cultivars grown in different environment.

Materials and Metods

The variability of grain harvest index (GHI) and some yield components (plant height -PH, thousand grain weight-TGW) were studied in 20 wheat cultivars originated from different wheat breeding centers. For this investigation used cultivars: Balkan, NS Rana 2, Jugoslavija, Yantar, Rusalka, Šumadinka, Maris Freeman, Zadruga, Kompas, Gružanka, Apache, KG 78, Lepenica, Zastava, Gruža, Lasta, Libellula, Ljuičevka Partizanka and Pobeda. The experiment was performed in randomized block design in five replication on the experimental basic plot 5 m^2. For analysis of plant height, were used 100 plants in full maturity stage (20 plants/replication). The following parameters were computed: the average value (x); the variance (σ^2); the coefficient of variation (V) as an index of relative variability of the trait. The significant differences between the average value were estimated by F-test values. The differences of wheat cultivars was estimated on the base of analyzed parameters: harvest index, plant height, thousand grain weight.

Climatic conditions during growing seasons

The values of temperature and precipitation in two years experiment were different and compared with previous ten years average values (table 1).

Table 1. Monthly and mean temperatures and monthly and cumulative precipitation

Tem& Precpt	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
^{0}C	2005/06	11.5	5.6	3.3	-1.7	1.5	5.5	12.7	16.4	19.7	8.3	74.4
^{0}C	2006/07	13.3	7.6	3.5	6.1	6.3	9.1	12.1	18.2	22.8	11.0	99.0
1990/2000		11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	8.5	76.7
(mm)	2005/06	49.0	54.8	47.1	27.9	38.1	116	86.3	29.6	84.8	59.3	533.7
(mm)	2006/07	16.7	13.7	51.9	45.3	32.1	62.9	3.6	118	25.3	41.1	369.9
1990/2000		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.5	418.5

Average temperatures were similar during ten years average value (8.5 ^{o}C) and first year (8.3 ^{o}C) of investigation, while in second year temperature value (115 ^{o}C) was higher in average about 2.5 5 ^{o}C than in the first (2005/06) and ten year period (1990/2000). Sums of precipitation were higher in 2005/06 (533.7mm) than in 2006/07 (369.9mm) year and ten year precipitation amount (418.5mm). According to long-term period, precipitations in 2006/07 vegetative period were lower for 115.9 mm, while 2005/06 amount of precipitation was higher for (47.9mm). In May 2006/07 precipitations were higher four time in relation to 2005/06 year and long-term period, but in April 2006/07 was only 3.6 mm.

Results and Discussion

Harvest index (HI). For small grains, harvest index (HI) is the ratio of weight of harvested grain to total shoot dry matter and represents plant efficiency in translocation nutritive matter from vegetative to generative plant part. Harvest index value can be used as a measure of efficiency of productivity. GHI depends to two distinctly quantitative traits contribute to high variability of cultivars. In the first year of investigation the GHI value ranged from $\bar{x}$ = 35.10% for cultivar Zastava to $\bar{x}$ = 41.30%, for Lasta cultivar. In second year harvest index value variate from $\bar{x}$ =33.20% in cultivar Ljubičevka to $\bar{x}$ =42.60% in Pobeda cultivar (tab. 2). Variability expressed by coefficient of variability in first year (V=5.23%) and in second year (V=5.84%).

Table 2. Average value of analyzed harvest index, plant height and thousand grain weight in wheat cultivars in period 2005-2007 years

Cultivar	Plant height (cm)		1000 grain weight (g)		Harvest index (%)	
	2005/06	2006/07	2005/06	2006/07	2005/06	2006/07
Balkan	80.15	63.54	45.35	50.15	38.8	37.4
NS Rana 2	80.52	66.36	39.83	40.90	38.6	40.6
Jugoslavija	84.68	69.24	47.80	46.10	38.9	38.6
Yantar	75.43	57.38	40.18	42.25	39.4	41.4
Rusalka	71.20	58.18	40.14	41.86	37.6	39.8
Šumadinka	75.98	60.84	38.80	41.10	38.9	40.2
Maris Freeman	80.20	65.10	42.10	42.88	36.8	39.2
Zadruga	89.15	74.12	51.12	52.85	39.1	41.6
Kompas	75.81	71.56	42.30	45.52	40.6	41.0
Gružanka	76.42	63.25	38.33	39.50	38.8	39.4
Apache	86.54	70.06	44.60	44.16	38.7	39.9
KG-78	81.52	65.68	43.20	41.30	38.9	39.0
Lepenica	82.16	62.15	41.36	38.84	37.8	37.1
Zastava	98.32	78.84	46.14	44.26	35.1	35.8
Gruža	70.56	57.10	42.20	47.55	39.1	40.1
Lasta	75.90	58.32	44.33	44.68	41.3	41.9
Libellula	75.21	66.44	45.35	45.88	37.4	37.5
Ljubičevka	85.80	72.05	41.84	44.65	35.2	33.2
Partizanka	84.76	67.24	42.80	41.90	38.7	41.0
Pobeda	81.10	65.82	43.12	45.20	40.2	42.6
V %	5.12	4.46	3.08	4.32	5.23	5.84
$LSD_{0.05}$	1.936	1.624	0.806	0.852	0.806	0.808
$LSD_{0.01}$	2.623	2.169	1.063	1.122	1.062	1.066

Harvest index is reliable indicator of yield and significant correlation between harvest index and grain yield were found for 13 wheat cultivars of USA (Donmez et al., 2001), for 14 wheat cultivars of France (Brancourt-Hulmel et al., 2003) and for UK wheat varieties (Shearman et al., 2005). The negative correlation between harvest index and protein content in wheat were found Flood and Martin (2001) what is indicate difficulties to create cultivar with high yield and high quality. This could be connected with weather conditions, which causing shortening of stem, that was favorable for nutritive matter translocation. Predicting or modeling crop yield or harvest index responses to temperature extremes during the reproductive phase is problematic due to differences among genotypes, and difficulties to estimate damages of grain

by extreme temperature. The analysis of variance indicated that environmental conditions had significant influence on expression harvest index value. The efficiency with which wheat plant produce dry matter of grain is the first determinant of harvest index. For the most wheat cultivars the harvest index is below the 50%. The achieving high value of HI based on genotype capacity and value of adaptability to environmental factors as well variation of temperature, carbon concentration, soil fertility, water and nutrient availability.

Plant height. This trait is related to grain harvest index and grain yield in wheat cultivars. The plant height in the first year of investigation the ranged from $\bar{x}$ = 70.56cm for cultivar Gruža to $\bar{x}$ = 98.32cm, for Zastava cultivar. In second year plant height value varies from $\bar{x}$ =57.10cm to $\bar{x}$ =78.84cm (Table 2). Variability expressed by coefficient of variability in first year (V=5.12%) and in second year (V=4.46%) tab. 2. The differences are most likely caused by genetic diversity of studied wheat cultivars and environmental conditions in which field experiment was performed. Plant height controlled by genes and environment. The decreasing of plant height can contribute to increasing value of harvest index. *Rht* genotype had a significant effect on reduction plant height, coleoptile length, and yield. For the semi-dwarf wheat genotypes were found enhanced interaction with environment in comparison to tall cultivars (Dimitrijevic et al., 2000). The wheat cultivars with decreased stem height have increased efficiency of utilization mineral elements and grain filling. The genes *Rht-B1b, Rht-D1b* mainly have effect to plant height reductions about 20% and increasing of grain yield potential for 5-15%, while *Rht8* influence to stem reduction about 10% and decrease yield for about 5% (Robbins, 2009).

Thousand grain weight. In the first year, the highest average value of thousand grain weight had Zadruga cultivar (x=51.12g) the lowest average value of thousand grain weight expressed Gružanka cultivar (x=38.33g) and Šumadinka (x=38.80g). In second year of investigation the highest value of TGW had Zadruga cultivr (52.85g) and the least value of TGW had Lepenica cultivar (38.84g).Variability of thousand grain weight in analyzed cultivars, in average, was expressed and coefficient of variation had (V=3.08%) in the first year and (V=4.31%) in the second year (tab. 2). TGW have strong influence to spike productivity and yield value. For analyzed traits the environmental differences contributed more to total trial sum of squares than genotypic diversity. The biological yield, harvest index and TGW contribute to increasing of yield (Tsegaye et al., 2012) and that this trait, depends of phase of development variate under environmental factor (Khan and Dar, 2010) on the both phenotypic and genotypic levels (El-Moshen et al., 2012).

Conclusion

This study showed variability of harvest index, plant height and thousand grain weight and differences among the investigated wheat cultivars. Difference among analyzed wheat cultivars were significant and determined by genotype specificity and environmental factor. The highest grain harvest index in first year had cultivar Lasta (41.3%) and in the second year had cultivar Pobeda (42.6%). Other cultivars had different value of HI in two different environment. This difference values of HI in analyzed wheat cultivars indicated that remobilization of reserve into harvested organs varies among the cultivars. Cultivars expressed different reaction in plant height reducing, consequently that had the impact on HI, as well. Wheat cultivar Zastava had the highest height (98.32cm) in first and 78.84cm in second year and low values of HI in both year. The best wheat cultivar for TGW was Zadruga (51.12g) in the first year and 52.85g in the second year of investigation. The values of GHI, plant height and TGW was different among the investigated wheat cultivars as well for each cultivar depends of year of investigation, which indicated different response of genotype to environmental conditions in two experimental years.

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GERMINATION OF MAIZE HYBRIDS SEED STORED AFTER HARVEST

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Abstract

In this investigation used three maize hybrids that are belongs to different group of ripening: FAO 3, FAO 4 and FAO 6. The seed of those hybrids used for investigation of genotypic divergences for seed germination, energy of germination, content of abnormal emergence of seeds, content of dead seeds. Hybrid seed for analysis produced on the experimental field of the Institute of Maize in Zemun Polje, Belgrade. The viability of the maize hybrids seeds were determined in experimental analysis carried out in laboratory for quality of seed in the Maize Research Institute Zemun Polje, Belgrade. According to standard method on filter paper were estimated the values of seed germination. The three experiment of seed germination carried out: 2 months after harvest, 9 months after harvest and 18 months after harvest. In average for each test the highest percentage of seed germination were found for hybrid ZP FAO3: 92.75% for seed after 2 months of harvest, 92.00% for seed 9 monhts after harvest and 91.50% for seeds after 18 months of harvest. This hybrid had the lowest percentage of ungerminated seeds (2.75%) and the least content of dead seed (~5.00%). The lowest value of seed germination (86.33%) and the highest values of ungerminated seed (~3.00%) as well the highest content of dead seed (10.33%) had hybrid belongs FAO 4 group. The obtained results indicate differences of germination in analyzed maize genotypes.

Key words: *seed, germination, percentage, hybrid, maize*

Introduction

Value of seed germination is very important trait of genotypes and represent initial stage of plant development. The plant species potential for reproduction is determined with seed germination. Genetic potential for seed germination is different among cultivars and hybrids and expresed in interaction with environmental conditions. The process of seed germination require available water which is need for activating enzyme system and initiating growth and development of seedlings (ISTA 2010). Seed germination is the most important trait of quality and life cycle of seeds, depends of genotypes and environments, which determine efficiency of plant growth (Milošević and Malešević, 2004). In the aim to establish value of seed germination, developed different methodes for analysis of seed germination of different plant species as well as for maize (Milošević et al., 1994). The identification of quality of seed contribute to undestand its importance for economy. International seed trade represents one of the basic indicators of an economic status of a country. Seed production is among the most profitable activities in the field of agriculture, considering relation of areas under commercial seed production and high financial effect (Knežević et al., 2006). Methods for germination of maize seeds on filter paper and sand represent standard test under optimal humidity of supstrate, as well temperature and humidity of the environment. This methods used filter paper is very short and efficient for establishing of seed germintion. The standard test of germination healthy and unharmefull seeds under favorable laboratory condition give us useful information about seed germination capacity (Milošević et al., 2007). However,

standard tests, often do not show realistic behavior of the seed under field conditions are still used. In the case of high doses of treatment by pesticides can use larger fraction of seeds for testing in sand. In purpose to establish seed germination for planting in cold field conditions used different type of vigor tests. Germination percentage of seeds in laboratory will give information that seeds can planted in field conditions and develop into normal plants. The growing of maize hybrids in different environment can be associated with variations in seed germination ability under the influence of environmental limiting factors as well soil fertility, climate (Kovačević et al., 2011; Ranieri et al., 2012). Several ecological and evolutionary factors can affect the process of seed germination, as well seed size which have an important evolutionary effects on plant reproduction of many plant species (Moles et al., 2006), and have influence to germination time, germination percentage and seedling vigor (Yanlong et al., 2007).

The aim of this work is study of variability of seed germination hybrids belongs to different maturity group and established differences of percentages of germinated seeds in laboratory conditions.

Material and Methods

The three maize hybrids, originated from Institute of maize Zemun Polje, Serbia (ZP-FAO 3. ZP-FAO 4 and ZP-FAO 6) were utilized for investigation. Four replicates of hundred seeds of each hybrids were used in laboratory tests. The standard germination test was conducted in rolled paper towels placed in germinator at 20°C (16 hours) and then at 30°C (8 hours) according to the rules of the International Seed Testing Association (2010). The germinator was set to provide light during the high-temperature cycle (8 hours) and to remain dark during the low-temperature cycle (16 hours). An initial count of germination percentage was 5^{th} day and a final count 7^{th} day after beginning of germination test.

For data analysis was taken Kruskall-Wallis's test, which is a non-parametric alternative one way of analysis of variance with different patterns. Results are converted into ranks, and comparing the middle ranks of each group. Statistical analysis was performed using the "R programming language" (The R Project for Statistical Computing, version 3.1.3) (R Development Core Team, 2015).

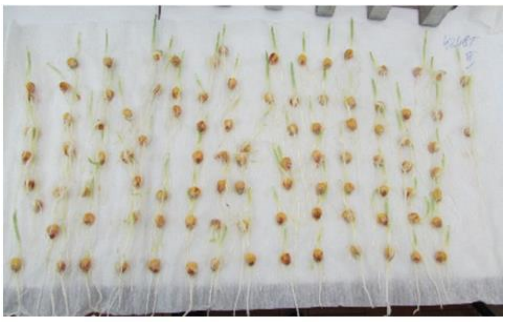
Results and Discussion

In this investigation obtained results showed variability of seed germination in laboratory conditions depends on hybrids and different length of storage time after harvest. The lowest value (86.10%) of seed germination energy, in average had the hybrid ZP FAO 4 and the highest germination energy (91.60%) had hybrid ZP FAO 3. Also in all test of grmination, the highest seed germination energy had ZP FAO 3 in test of two months after harvest - 92.25%, nine months after harvest-91.50% and 18 months after harvest 91.25%. The lowest energy of seed germination had hybrid ZP FAO 4, in test at two months after harvest - 86.75%, nine months after harvest-85.00% and 18 months after harvest 86.50%. Seed germination energy of hybrid ZP FAO 6 was in test at two months after harvest -86.50%, nine months after harvest-91.00% and 18 months after harvest 91.50% (table 1).

Table 1. Percentage of seed germination energy and percentage of seed germination

	Seed germination energy % after harvest			Average %	Value of seed germination % after harvest			Average %
Hybrid	2-months	9-months	18-months		2-months	9-months	18-months	
ZP FAO 3	**92.25**	**91.50**	**91.25**	91.60	92.75	92.0	91.50	92.08
ZP FAO 4	**86.75**	**85.00**	**86.50**	86.10	**87.50**	**85.75**	87.25	86.33
ZP FAO 6	**86.50**	**91.00**	91.50	89.66	**93.25**	**91.75**	90.25	91.75

The lowest value of seed germination (86.33%), in average had the hybrid ZP FAO4 and the highest value of germination (92.08%) had hybrid ZP FAO3. Also in all test of grmination, the highest percentage of seed germination had ZP FAO3, two months after harvest -92.75%, nine months after harvest-92.0% and 18 months after harvest 91.50%. The lowest seed germination had hybrid ZP FAO4, in test at two months after harvest -87.50%, nine months after harvest-85.75% and 18 months after harvest 87.25%. Seed germination energy of hybrid ZP FAO6 was in test at two months after harvest -93.25%, nine months after harvest-91.75% and 18 months after harvest 90.25% (table 1, picture 1.).
Data about differences between maize hybrids for seed germination reported in investigation of other genotypes (Milošević et al. 1994) depends of endosperm of seeds (Pajić et al., 1998) and in different environmental conditions (Meeks et al., 2013).



Picture 1. Test of seed germination in maize hybrids

Picture 2. Occurrence of abnormal seed germination and dead seeds

Table 2. Content of abnormal seeds and dead seeds of maize ZP hybrids

	Abnormal seed germination (%) after harvest			Average	Dead seed at germination % after harvest			Average
Hybrid	2-months	9-months	18-months		2-months	9-months	18-months	
ZP FAO 3	**2.75**	**2.75**	**2.75**	2.75	**4.50**	**5.25**	**5.25**	5.00
ZP FAO 4	**3.75**	**3.50**	1.75	3.00	**9.00**	**11.0**	11.0	10.33
ZP FAO 6	**2.25**	**2.25**	2.75	2.75	**4.50**	**6.00**	7.00	5.82

During the analysis of seed germination, abnormal germination of seed occured, and dead seed (picture 2). The value of abnormal seed germination was similar for the investigated maize hybrids, approximately 3% (table 2) However, the lowest percentage of dead seed had hybrid ZP FAO3 (5.00%) and ZP FAO6 (5.82%), while the highest content of dead seed had hybrid ZP FAO4 (10.33%) table 2.
The results of seed germination obtained by using standard method in the filter paper showed that the values obtained in three term after harvest are very few deviate for one hybrids, but there was differences between values obtained in the same test term after harvest for each analyzed hybrid of maize. For seed energy of germination and germination of seed were found differences between analyzed maize hybrids. The obtained values of χ^2 =1.96 for germination energy of seeds is with probability 0.5499 (figure 1), while for percentage of seed germination influence of genotype for computed χ^2 =0.11 is with probability 0.9465 (figure 2). On the base of obtained values of probability on the level of >0.05 differences between genotypes are not significnt.

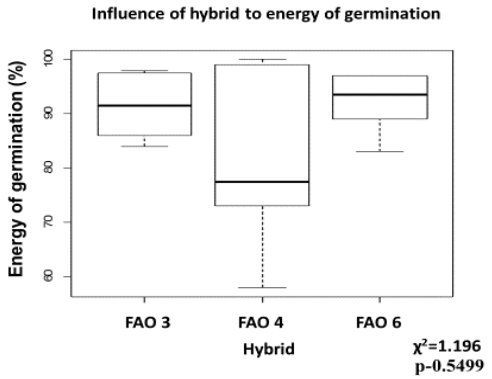


Figure 1. Effect of hybrids to germination energy

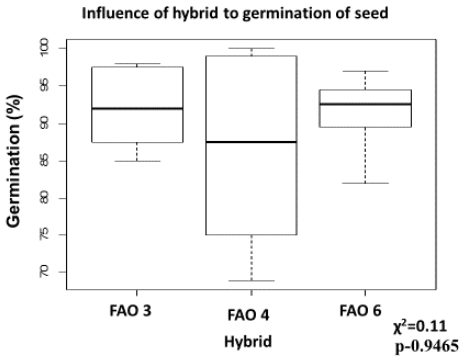


Figure 2. Influence of hybrids to germination

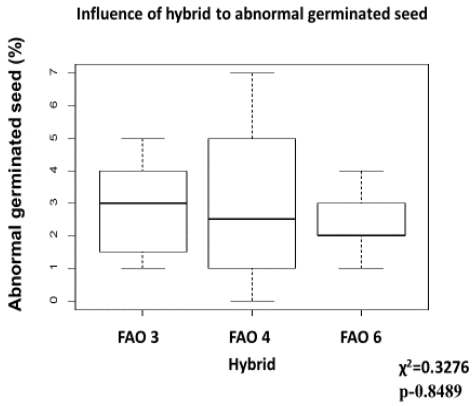


Figure 3. Effect of hybrids to occurence of abnormal seed germination

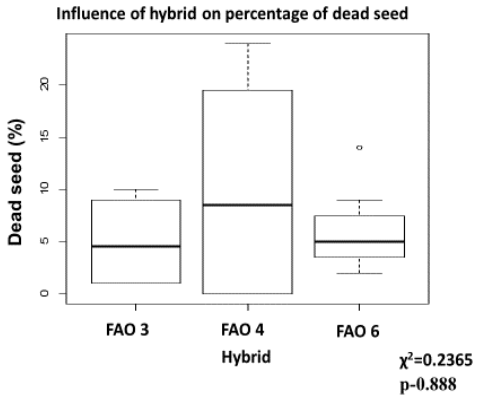


Figure 4. Influence of hybrids to occurence of dead seed

The differences between hybrids were not significant for occurence abnormal seed germination have probability 0.8489 for obtained values of χ^2 =03276 (figure 3) and for dead seed obtained values of χ^2 =0.11 was with probability 0.888 (figure 4).

Table 3. Kruskal-Wallis test for effect of seed storage time on traits of seed test germination in maize hybrids

Trait	χ^2	DF	p	Trait	χ^2	DF	p
Energy of germination	0,5888	2	0,745	Abnormal seeds	0,0378	2	0,9813
Percentage of germination	0,3464	2	0,841	Dead seed	0,4121	2	0,8138
χ^2-Chi square; DF-degree of freedom; p-probability							

The estimation of influence of different length of storage time after harvest on energy of seed germination, percentage of germination, content of abnormal germination of seeds and dead seeds presented in table 3. The obtained values of probability at level >0.05 for those traits indicate that included periods of storage seed after harvest do not have significant influence on seed germination. Storage time did not affect the values of seed germination, indicating that the seed is vital and that storage conditions were appropriate.

Conclusion

In this study were found differences among hybrids for seed germination energy, seed germination, occurence of abnormal seed germination and dead seed. In average the highest

germination energy (91.60%) and germination of seed (92.08%) had ZP FAO3, and the lowest germination energy-86.10%, and germination-86.33% had maize hybrid ZP FAO4. The occurence of abnormal seed germination was the lowest (2.75%) in hybrid ZP FAO3. The lowest content of dead seed established in ZP FAO3 (5.00%) and the highest in ZP FAO4 (10.33%). Included length of storage time after harvest of seed did not have significant influence on expressed differences of seed germination which were stored in appropriate conditions. Germination testing in the laboratory is done under very favorable, controlled conditions (humidity, temperature, light). Such results can be a good indicator of seed germination in field conditions where they are usually unfavorable factors as temperature and humidity, the structure of soil and soil pests.

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INFLUENCE OF TEMPERATURE ON POLLEN GERMINATION AND POLLEN TUBE GROWTH OF PLUM CULTIVARS

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Abstract

The temperature is an important environmental factor that affects pollen performance during the reproductive phase of fruit tree species. Pollen germination is one of the main factors for successful fertilization and fruit set in plum (Prunus domestica L.). The study was carried out to determine the effect of three different temperatures (5, 15 and 25°C) on the pollen germination and pollen tube growth in vitro of six European plum cultivars: ‘Cacanska Lepotica’, ‘Cacanska Rana’, ‘Jojo’, ‘Top’, ‘Toper’ and ‘Stanley’. Germination rate and pollen tube growth were determined on a culture medium containing 15% sucrose and 0.7% agar. Temperature significantly affected pollen germination of all studied cultivars. High germination rates (50–76%) were obtained at the temperatures of 15°C and 25°C. However, satisfactory germination rates (29–47%) were also obtained at the temperature of 5°C in some cultivars (‘Toper’, ‘Cacanska Rana’, ‘Cacanska Lepotica’ and ‘Top’). The influence of temperature was more prominent on the pollen tube growth. The length of pollen tubes was six to twelve times higher at 15°C and 25°C in comparison with 5°C. This has led to the conclusion that the temperature of 5°C, although it could be enough for pollen germination, is not enough for optimal pollen tube growth.

Keywords: *Prunus domestica*, pollen germination *in vitro*, pollen tube length.

Introduction

Pollen viability and its germination capacity is one of the main factors for successful fertilization in fruit trees. It is important for both breeding work and choice of the varietal composition in orchards.

Parfitt and Ganeshan (1989) studied seven different tests to evaluate pollen viability in several species of the genus *Prunus.* They concluded that two *in vitro* germination tests (hanging-drop and agar-plate) were the most reliable.

Temperature is the most important environmental factor affecting the success of fertilization and fruit set of stone fruit species. It affects different stages of a reproductive process, such as a stigmatic receptivity (Hedhly et al., 2003; Hedhly et al., 2005), pollen germination (Keulemans, 1984; Egea et al., 1992; Pirlak, 2002; Hedhly et al., 2004), pollen tubes growth (Cerović and Ružić, 1992; Hedhly et al., 2005; Milatović and Nikolić, 2014), and ovule viability (Stöser and Anvari, 1982; Postweiler et al., 1985; Cerović et al., 2000).

Keulemans (1984) examined the effect of six different temperatures (4, 6, 9, 12, 15 and 18°C) on pollen germination and pollen tube growth of six plum cultivars. The examined cultivars had different reaction on temperature. Cultivars ‘Czar’, ‘Opal’ and ‘Victoria’ were characterized by good germination and fast growth of pollen tubes at low temperatures.

The aim of this study was to determine the effect of temperature on pollen germination and pollen tube growth in six cultivars of European plum.

Materials and methods

Six plum cultivars: ‘Cacanska Lepotica’, ‘Cacanska Rana’, ‘Jojo’, ‘Top’, ‘Toper’ and ‘Stanley’ were used as a material for testing the pollen performance. Plant material was taken from a collection orchard of the Experimental farm “Radmilovac” of the Faculty of Agriculture in Belgrade. The orchard was planted in 2009. The rootstock was Myrobalan (*Prunus cerasifera* Ehrh.) seedlings, the crown form was Central Leader, and tree spacing was 4.5 × 3 m.

To collect pollen samples, twigs with flower buds in the “balloon” stage were taken from the experimental orchard. The twigs were transported to the laboratory where anthers were isolated from the flower buds. They were left to desiccate and release pollen in open Petri dishes for 24–48 h. Pollen was sowing using a fine brush in Petri dishes on previously prepared medium which consisted of 0.7% (w/v) agar-agar and 15% (w/v) sucrose. Petri dishes were then transferred to incubators ‘FOC 225I’ (Velp Scientifica, Usmate, Italy) at three different temperatures: 5, 15 and 25°C. After incubation of 24 hours, 40% (v/v) formaldehyde was added in Petri dishes to prevent further growth of pollen tubes.

Petri dishes with sowed pollen were observed under light microscope ‘Leica DM LS’ (Leica Microsystems, Wetzlar, Germany). The number of germinated and non-germinated pollen grains was counted and the percentage of germination was calculated. Petri dishes were divided into three parts, each part representing one repetition. In each repetition at least 300 pollen grains were analysed. Pollen was considered germinated if the length of the pollen tube was greater than the diameter of the pollen grain.

Pollen tube length was measured in pictures taken under the microscope ‘Leica DM LS’ using the ‘Leica IM 100’ programme. From all experiment variants (cultivars and temperatures) 60 pollen tubes were measured.

The experiment was conducted as two-factorial (cultivar, temperature), with three repetitions. The data were statistically analysed using the analysis of variance. Percentage data were subjected to arcsine square root transformation before the statistical analysis. Duncan’s multiple range test (5%) was performed for comparing means.

Results and discussion

Pollen germination ranged from 15.4% in the cultivar ‘Jojo’ at the temperature of 5°C to 76.3% in the cultivar ‘Cacanska Lepotica’ at the temperature of 25°C (Figure 1). In all studied cultivars pollen germination was significantly lower at the temperature of 5°C than at higher temperatures (15 and 25°C). Differences in pollen germination between temperatures of 15°C and 25°C were not statistically significant.

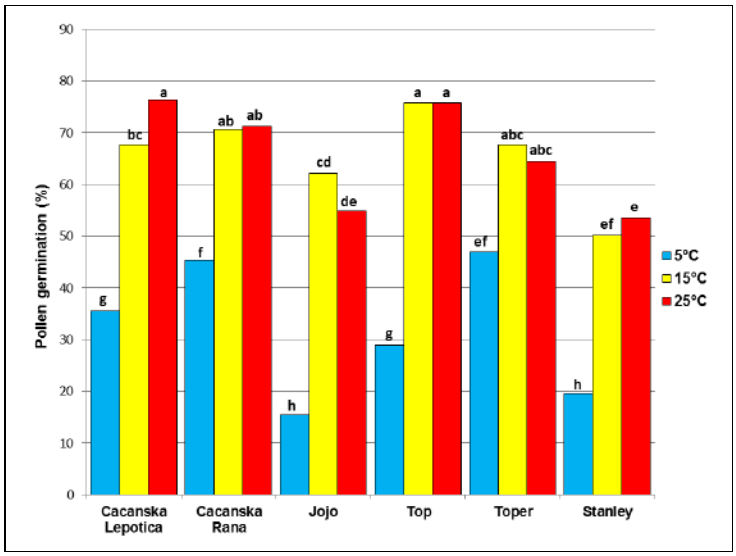

Figure 1. The influence of temperature on pollen germination of plum cultivars.

Differences between the cultivars were also significant (Table 1). Cultivars ‘Cacanska Lepotica’, ‘Cacanska Rana’, ‘Top’ and ‘Toper’ have higher germination rates (60-62% in average for all temperatures) comparing to cultivars ‘Jojo’ and ‘Stanley’ (41-44% in average).

Table 1. Analysis of variance for pollen germinatin and pollen tube length.

Source of variation	Pollen germination		Pollen tube length	
	df	Mean squares	df	Mean squares
Cultivar (G)	5	45.57**	5	2.12$^{n.s.}$
Temperature (T)	2	430.50**	2	437.91**
G x T	10	9.64**	10	1.55$^{n.s.}$
Error	36	17.07	36	125.477,4

**: statistically significant at 0.01 probability level; $^{n.s.}$ non-significant.

Cultivars ‘Toper’, ‘Cacanska Rana’, ‘Cacanska Lepotica’ and ‘Top’ had significantly higher germination rates at the temperature of 5°C compared to ‘Jojo’ and ‘Stenley’. This could point to their better adaptation to colder weather conditions during flowering.

Previous research has found that pollen germination depends on the genotype. Different authors have found great variation in the degree of pollen germination *in vitro* in European plum cultivars: Botu et al. (2002) 12-61%, Surany (2006) 25-64%, Koskela et al. (2010) 3-70%, Sharafi (2011) 46-72%, Nikolić et al. (2012) 30-67%. The results obtained in this study are within the specified values.

According to the states of Wertheim (1996) that pollen germination of 25% is considered as a threshold for satisfactory germination in plum, it can be said that the studied cultivars are characterized by good pollen germination rates. Therefore, they can be recommended as potential pollenizers for other cultivars.

The minimum length of pollen tubes was obtained in cultivar ‘Jojo’ at the temperature of 5°C and it was 127 µm, while the maximum length was obtained in ‘Stanley’ at 25°C and it was 1727 µm (Figure 2).

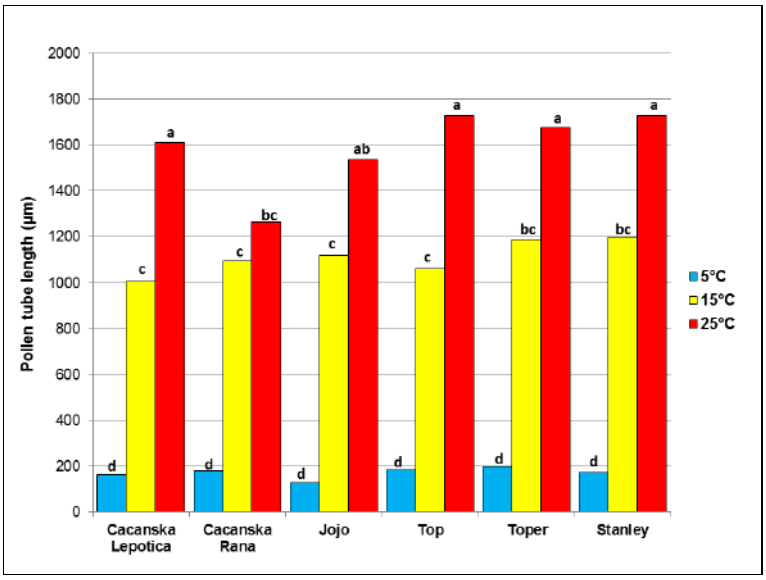

Figure 2. The influence of temperature on pollen tube length of plum cultivars.

The average values of pollen tubes length were the highest at 25°C (1589 µm), then at 15°C (1111 µm), while they were the lowest at 5°C (170 µm). The length of pollen tubes at temperature of 15°C was significantly higher than at the temperature of 5°C in all studied cultivars. Also, the length of pollen tubes at temperature of 25°C was significantly higher than at the temperature of 15°C in all cultivars, except ‘Cacanska Rana’. Differences between cultivars, as well as interaction Genotype × Temperature were not statistically significant (Table 1).

The average values of pollen tube length obtained in this study were higher than the values obtained by Sharafi (2011). Increasing of the temperature resulted in a significant increase in the length of pollen tubes, which confirms the results of previous studies (Cerović and Ružić, 1992; Pirlak, 2002; Hedhly et al., 2004; Milatović and Nikolić, 2014).

Conclusion

Temperatures of 15 and 25°C are optimal for pollen germination and pollen tube growth of European plums. However, the rates of pollen germination at 5°C were higher in some cultivars (‘Toper’, ‘Cacanska Rana’, ‘Cacanska Lepotica’ and ‘Top’) than in others (‘Jojo’ and ‘Stanley’). Therefore, these cultivars could be better adapted to the low temperatures during flowering.

The influence of temperature was more prominent on the pollen tube growth. The length of pollen tubes was six to nine times higher at 15°C and seven to twelve times higher at 25°C in comparison with 5°C. This leads to the conclusion that the temperature of 5°C, although it could be enough for pollen germination, is not enough for optimal pollen tube growth.

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PHENOLOGICAL CHARACTERISTICS, YIELD AND FRUIT QUALITY OF INTRODUCED APRICOT CULTIVARS IN THE REGION OF BELGRADE (SERBIA)

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Abstract

The evaluation of ten introduced apricot cultivars originated from Romania, Bulgaria and Hungary was carried out in the region of Belgrade over a period of six years (2009–2014). Control cultivar for comparison was 'Hungarian Best'. Flowering of introduced cultivars was from two days before to two days after the control, while harvest was from 6 days before ('Neptun') to 13 days after ('Litoral'). The average yield per tree ranged from 5.7 kg in 'Kasna Drjanovska' to 12.2 kg in 'Mari de Cenad' and 'Roksana', while the average fruit weight ranged from 45.1 g in 'Umberto' to 82.0 g in 'Neptun'. Compared with the control cultivar, significantly higher yield was achieved in seven cultivars, while significantly higher fruit weight was found in six cultivars. The content of soluble solids varied from 14.2% in 'Cegledi arany' to 19.5% in 'Kasna Drjanovska'. Cultivars 'Roksana', 'Mari de Cenad', 'Gergana' and 'Neptun' stand out for fruit appearance, and 'Silistrenska kompotna', 'Kasna Dryanovska' and 'Mari de Cenad' for fruit quality. Among studied cultivars, for growing in this region 'Dacia', 'Gergana', 'Mari de Cenad', 'Neptun' and 'Roksana' can be recommended, predominantly for fresh fruit consumption. In addition, cultivars 'Silistrenska kompotna' and 'Kasna Drjanovska' can also be recommended, mostly for fruit processing.

Key words: *Prunus armeniaca*, flowering, maturing, yield, fruit, stone

Introduction

Extensive research is conducted worldwide on the creation of new apricot cultivars with improved characteristics, such as better adaptability to different environmental conditions, higher resistance to disease-causing agents, higher yield, and better fruit quality. In the last 20 years, more than 500 new apricot cultivars were created. The largest number of new cultivars was created in the United States, followed by France, Italy, Romania, China, Czech Republic and Spain (Milatović, 2013a).

Apricot cultivars have limited ecological adaptability. Thus, the leading cultivars are different in each region of production. Due to the limited adaptability, the best results in the apricot production are achieved with cultivars bred in the similar environmental conditions.

Romania is one of the leading countries in apricot breeding with more than 40 new cultivars released. Breeding has conducted in fruit research stations in Mărculești, Băneasa, Constanţa and Oradea (Cociu, 2006; Bălan et al., 2010; Topor et al., 2010). In Bulgaria, apricot breeding is conducted in Experimental Station Silistra and Fruit Growing Institute in Plovdiv (Tsoneva and Tsonev, 1995; Lyubenov, 2005; Zhivondov, 2012). In Hungary, 20 new apricot cultivars are bred, and the leading institutions are Fruit Research Station Cegled and Faculty of Horticulture, Corvinus University of Budapest (Szalay et al., 2005; Pedryc and Hermán, 2012).

The aim of this study was the evaluation of ten introduced apricot cultivars originating from Romania, Bulgaria and Hungary. The best performing cultivars will be recommended for growing in the region of Belgrade, as well as in other regions with similar ecological conditions.

Material and methods

The study was conducted in the apricot collection orchard at the Experimental Station "Radmilovac" of the Faculty of Agriculture in Belgrade during the period of six years (2009–2013). The orchard was planted in 2007. The rootstock is Myrobalan (*Prunus cerasifera* Ehrh.) seedling, training system is central leader, and planting distance is 4.5 x 3 m. All cultivars are represented by five trees.

The study included ten apricot cultivars: five from Romania ('Dacia', 'Litoral', 'Mari de Cenad', 'Neptun' and 'Umberto'), four from Bulgaria ('Gergana', 'Kasna drjanovska', 'Roksana' and 'Silistrenska kompotna') and one from Hungary ('Cegledyi arany'). 'Hungarian Best' cultivar was taken as a control.

Flowering was recorded by recommendations of the International Working Group for pollination: start of flowering – 10% open flowers, fool bloom – 80% open flowers, end of flowering – 90% of the petal fall (Wertheim, 1996). Trunk cross-sectional area (TCSA) was calculated on the basis of trunk circumference measured at the height of 30 cm above the ground level. Cumulative yield efficiency was calculated by dividing the cumulative yield over six years by the TCSA in the last year (2014). Fruit characteristics were measured on a sample of 25 fruits per cultivar. Fruit shape index was calculated using the formula: length × length / width × thickness. Soluble solids were determined by refractometer and total acids (expressed as malic acid) by titration with 0.1 N NaOH. Sensory characteristics of the fruit (appearance and taste) were evaluated by a five-member jury, scoring the cultivars using the scale from 1 to 5 points.

The obtained data were statistically analyzed using analysis of variance. The significance of differences between mean values was determined using Tukey's test at 0.05 level of probability.

Results and discussion

Average time of flowering of apricot cultivars was late March and early April (Table 1). Among studied cultivars small differences in flowering time were recorded. The start of flowering was from two days before the 'Hungarian Best' ('Litoral') to two days after the control ('Cegledi arany', 'Kasna Drjanovska', 'Umberto' and 'Silistrenska kompotna'). The longest duration of flowering was recorded in 'Neptun' and 'Roksana' cultivars.

Table 1. Phenological characteristics of apricot cultivars (average, 2009–2014).

Cultivar	Flowering dates			Duration of flowering	Harvest date	No. of days comparing to control
	Start	Full	End			
Cegledi arany	29.03.	31.03.	05.04.	7.0	10.07.	+8
Dacia	26.03.	29.03.	03.04.	7.8	28.06.	–5
Gergana	26.03.	28.03.	01.04.	6.5	05.07.	+2
Kasna Drjanovska	29.03.	30.03.	05.04.	6.4	15.07.	+12
Litoral	25.03.	27.03.	02.04.	7.8	16.07.	+13
Mari de Cenad	26.03.	28.03.	02.04.	7.2	07.07.	+4
Neptun	27.03.	30.03.	05.04.	8.7	27.06.	–6
Roksana	27.03.	30.03.	04.04.	8.0	07.07.	+5
Silistrenska kompotna	29.03.	31.03.	06.04.	7.6	13.07.	+10
Umberto	29.03.	31.03.	05.04.	7.2	14.07.	+11
Hungarian Best (control)	27.03.	29.03.	03.04.	7.0	03.07.	0

Compared to the results of Milatović (2005) obtained at the same location for the ten-year period (1995–2004) duration of flowering was shorter by 2.8 days on average. This

difference can be explained by higher temperatures during flowering season in the period of study (2009–2014).

Average time of maturity was from 27th of June (‘Neptun’) to 16th of July (‘Litoral’). Compared to the control cultivar (‘Hungarian Best’) time of maturity was from 6 days before to 13 days after. Average difference in the date of maturity between the year with the earliest harvest (2009) and the year with the latest harvest (2010) was 8 days.

The average yield per tree ranged from 5.7 kg in ‘Kasna Drjanovska’ to 12.2 kg in ‘Mari de Cenad’ and ‘Roksana’ (Table 2). Compared with the control cultivar significantly higher yields were achieved in seven cultivars.

Table 2. Yield, trunk cross-sectional area (TCSA), and cumulative yield efficiency (CYE) of apricot cultivars.

Cultivar	Yield (kg per tree)							TCSA (cm^2)	CYE (kg/cm^2)
	2009	2010	2011	2012	2013	2014	Average		
Cegledi arany	0.1	5.3	1.7	8.5	14.9	27.6	9.7 ab	110.2 bc	0.53
Dacia	2.0	4.2	23.7	9.4	9.0	22.2	11.8 a	100.3 c	0.70
Gergana	0.2	2.2	11.5	12.1	0.1	24.2	8.4 abc	115.0 bc	0.44
Kasna Drjanovska	0.1	1.0	1.8	14.5	3.1	14.0	5.7 bc	133.8 b	0.26
Litoral	0.1	1.9	14.0	15.6	7.1	32.1	11.8 a	94.8 c	0.75
Mari de Cenad	0.6	7.7	24.3	12.8	0.5	26.9	12.2 a	97.0 c	0.75
Neptun	1.8	6.1	13.0	5.7	8.4	32.7	11.3 a	84.6 c	0.80
Roksana	0.8	6.8	16.7	11.4	1.3	36.3	12.2 a	85.8 c	0.85
Silistrenska kompotna	0.5	6.6	11.7	11.5	3.6	16.3	8.4 abc	147.2 a	0.32
Umberto	0.3	2.0	8.9	14.3	13.9	24.7	10.7 a	108.0 bc	0.59
Hungarian Best (control)	0.3	2.1	10.8	2.0	3.0	10.3	4.8 c	101.5 c	0.28

* Mean values followed by the same letter within a column do not differ significantly according to Tukey’s test at $P \leq 0.05$.

In most cultivars the highest yield was obtained in 2014 when the weather conditions were favourable. The lowest yield was recorded in 2013 because of the cold weather during the flowering which resulted in low fruit set (Zec et al., 2013). The obtained results of yield are in accordance with the results of Vachůn (2002), who studied the productivity of 24 apricot cultivars during six-year period and found variation of yield from 3 to 20 kg per tree.

Trunk cross-sectional area in two cultivars (‘Silistrenska kompotna’ and ‘Kasna Drjanovska’) was significantly higher than in the control cultivar. Cumulative yield efficiency ranged from 0.26 to 0.85 kg/cm^2. With the exception of ‘Kasna Drjanovska’, in all cultivars it was higher comparing to the control.

The average fruit weight ranged from 45.1 g in ‘Umberto’ to 82.0 g in ‘Neptun’ (Table 3). Compared to the control, significantly higher fruit weight was recorded in six cultivars.

Stone weight ranged from 2.9 g (‘Dacia’) to 4.7 g (‘Mari de Cenad’), and its share in the fruit weight ranged from 4.3% (‘Neptun’) to 8.7% (‘Silistrenska kompotna’). According to the classification given by Milatović (2013b) seven cultivars had small share in the fruit weight (under 5.50%). Two cultivars (‘Umberto’ and ‘Kasna Drjanovska’) had medium share of the stone in the fruit weight (6.51–8.50%), while one cultivar (‘Silistrenska kompotna’) had high share (above 8.51%).

Table 3. Fruit characteristics of apricot cultivars (average, 2009–2014).

Cultivar	Fruit weight (g)	Stone weight (g)	Stone share (%)	Fruit dimensions (mm)			Shape index
				Length	Width	Thickness	
Cegledi arany	65.1 c	3.7 d	5.7	48.3 b	50.3 ab	47.4 ab	0.98
Dacia	62.1 c	2.9 f	4.7	48.5 b	47.5 bc	44.8 abc	1.10
Gergana	70.8 b	4.5 ab	6.4	53.7 a	50.4 ab	46.4 ab	1.23
Kasna Drjanovska	55.7 d	4.5 ab	8.0	44.4 b	44.4 cd	43.8 c	1.01
Litoral	56.0 d	3.1 ef	5.5	54.1 a	45.0 cd	43.7 c	1.49
Mari de Cenad	81.8 a	4.7 a	5.7	57.2 a	53.1 a	48.9 a	1.26
Neptun	82.0 a	3.5 de	4.3	53.4 a	53.4 a	48.3 ab	1.11
Roksana	74.1 b	4.3 abc	5.8	55.4 a	50.8 ab	47.4 ab	1.28
Silistrenska kompotna	46.3 e	4.0 bcd	8.7	47.2 b	41.9 d	37.8 d	1.40
Umberto	45.1 e	3.1 ef	7.0	46.5 b	43.0 cd	41.7 cd	1.21
Hungarian Best (control)	51.7 d	3.8 cd	7.4	46.9 b	46.5 bcd	44.2 bc	1.07

* Mean values followed by the same letter within a column do not differ significantly according to Tukey's test at $P \leq 0.05$.

Fruit length of the studied cultivars varied from 44.4 to 57.2 mm, width from 41.9 to 53.4 mm, and thickness from 37.8 to 48.9 mm. Based on the fruit dimensions, the shape index was calculated, whose values ranged from 0.98 in 'Cegledi arany' (round flat shape) to 1.49 in 'Litoral' (elliptic shape).

Results of pomological fruit characteristics are in accordance with the previous findings for some cultivars (Cociu, 2006; Milatović et al., 2006; Korzin et al., 2010).

The content of soluble solids in tested cultivars varied from 14.2% in 'Cegledi arany' to 19.5% in 'Kasna Drjanovska' (Table 4). Cultivars 'Kasna Drjanovska' and 'Silistrenska kompotna' had significantly higher content of soluble solids than the control cultivar.

Table 4. Indices of fruit quality of apricot cultivars (average, 2009–2014).

Cultivar	Soluble solids (%)	Total acids (%)	Soluble solids /Total acids	Sensory evaluation (1–5)	
				Appearance	Taste
Cegledi arany	14.2 c	1.28 b	11.1	3.9 abcd	3.9 abc
Dacia	14.5 c	1.39 ab	10.5	3.9 abcd	4.0 ab
Gergana	15.5 c	1.25 b	12.4	4.2 abc	3.9 abc
Kasna Drjanovska	19.5 a	1.64 a	11.9	3.8 bcd	4.2 a
Litoral	14.9 c	1.66 a	8.9	3.5	3.4 c
Mari de Cenad	15.3 c	1.37 ab	11.2	4.4 ab	4.1 a
Neptun	16.1 bc	1.56 ab	10.4	4.2 abc	3.9 abc
Roksana	15.8 c	1.27 b	12.5	4.5 a	3.8 abc
Silistrenska kompotna	18.4 ab	1.35 ab	13.6	3.7 cd	4.3 a
Umberto	14.5 c	1.69 a	8.6	3.3	3.5 bc
Hungarian Best (control)	15.7 c	1.37 ab	11.4	3.6 cd	4.2 a

* Mean values followed by the same letter within a column do not differ significantly according to Tukey's test at $P \leq 0.05$.

The total acid content varied from 1.29% in ‘Gergana’ to 1.69% in ‘Umberto’. The ratio between soluble solids (consisting mostly of sugars) and acids contents indicates the sweetness of the fruit. It was the highest in cultivars ‘Silistrenska kompotna’ and ‘Roksana’ (more sweet taste) and the lowest in cultivars ‘Umberto’ and ‘Litoral’ (more acidic taste).

The data on the chemical composition of fruits are in accordance with the previous findings (Gurrieri et al., 2001; Ruiz and Egea, 2008).

Cultivars ‘Roksana’ and ‘Mari de Cenad’ stand out for attractive fruit appearance and got significantly higher scores than the control cultivar. The best scores for fruit quality got cultivars ‘Silistrenska kompotna’, ‘Kasna Drjanovska’ and ‘Mari de Cenad’. Cultivars ‘Litoral’ and ‘Umberto’ got significantly lower scores for fruit quality than the control cultivar.

Conclusion

Based on the six-year evaluation of ten introduced apricot cultivars in the Belgrade area, cultivars ‘Dacia’, ‘Gergana’, ‘Mari de Cenad’, ‘Neptun’ and ‘Roksana’ can be recommended for growing, predominantly for fresh fruit consumption. In addition, cultivars ‘Silistrenska kompotna’ and ‘Kasna Drjanovska’ can also be recommended, mostly for fruit processing.

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THE IMPACT OF ALFALFA CUTTING TERM ON NUMBER OF PLANTS

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Abstract

Alfalfa (*Medicago sativa* L.) is the most important forage plant in many regions of the world. The yield, quality and persistence of alfalfa depend on harvesting system and timely harvesting should ensure high yield and good quality of seed and forage crops and good persistence. The objective of this research was, in field conditions, in a model of dual utilization (seed, forage), to examine the effect of harvest term on the number of plants. The experiment was established in 2002 and the application of different harvesting systems started in next 5 year. The research included the first cut, as pre-cut to seed harvest, and it was harvested at different times – early harvesting, medium early harvesting, late and very late harvesting and harvesting system where two pre-cuts are harvested before seed harvest. The average number of plants decreased from 199 (second year) to 49 (in the sixth year). Growing conditions during some years and harvesting system had effect on the number of plants. In the first year of production, with denser density and extreme climatic conditions, reduction of the number of plants was greater than in the later years of exploitation and moderate conditions during the year. In the harvesting system with two pre-cuts, at the beginning there was a rapid decline in the number of plants.

Key words: *alfalfa, cutting term, plants*

Introduction

Economic importance of alfalfa is reflected in the production of high-quality forages, however, the seeds of alfalfa is valuable commodity in the domestic and international markets.Yield, quality and persistence of alfalfa depend on system of cutting (Sheaffer et al., 1988; Undersander et al., 2004). The system of cutting of alfalfa for forage production is used to harmonize the relationship between the yield of dry matter and content of nutrients in the plant to ensure maximum yield of nutrients per hectare. The system of harvesting for seed production should provide an optimal ratio of yield components of alfalfa seed. Flowering of seed crops should be in the preferable time, i.e. when the temperatures are high and the number and activity of the main types of insect pollinators the highest (Marble, 1989; Mueller, 2008).

By delaying the first cut in the system of seed production, when the second cut was used for seed production, the total energy production of NE_M and NE_L is decreased (Terzić et al., 2014). Contrary, delaying of the first cut can result in higher seed yield, when second cut was used for seed production (Karagić et al., 2007;Terzić, 2011).

Effect of harvest period on the persistence of the crop is pointed out by numerous authors (Karagić, 2004, Undersander et al., 2004, Terzić, 2011). Number of alfalfa plants is the highest in the year of establishing of crop and decreases with age. Thinning of alfalfa crop during the period of exploitation is a regular occurrence due to aging plants and effect of external factors, primarily low temperatures, heavy trampling caused by machinery, diseases and pests. In addition, the method of exploitation, i.e. the system of harvesting significantly influences the reduction of the number of plants. Too early harvesting exhausts plants, the

carbohydrate content in the root and the crown is lower than optimal, which reflects negatively on the yield and longevity of the crop (Sheaffer et al., 1988;Undersander et al., 2004).

Frequent cutting of undeveloped alfalfa (from the phase of vegetative growth to budding) or autumn cutting that prevents vegetative growth and development, as well as filling of root reserves, may lead to lower concentrations of non-structural carbohydrates in the root. This may be associated with the thinning and loss of yield. Earlier harvest will contribute to obtaining of high-quality feed, and later harvest will increase the reserves of the roots and persistence of the crop. The harvest in the fall is associated with persistence and yield (Undersander et al., 2004).

There has been lot of studies and activities worldwide focusing on the problem of finding an optimal solution in the seed production sector, however, there is no single uniform opinion. Most of the studies indicate that for each area must specific solutions must be determined.

The aim of this research is to examine the impact of different systems of cutting on number of plants in a combined mode of production (seed/forage).

Material and methods

Study was carried out on experimental field of the Institute for forage crops in Kruševac; Serbia.Size of the basic plot was 10.5 m^2. Treatments were different time of pre-cut of alfalfa seed crop: A_1- early cut (around May 5th), A_2- medium early cut (around May 15th), A_3- late cut (around May 25th), A_4- very late cut (around June 5th), A_5- cut (A5 I- around May 5th and II - around June 5th). Contrary to the harvest procedure in the A_1 to A_4 systems, where the second cut was used for seed production, in the A_5's harvest procedure the third cut was used for seed production. The final cut was done at the end of October. At the moment of cutting the plants were on average in the early system (A_1) in the stage of budding, in the medium early system (A_2), in the stage of the beginning of flowering, in the late (A_3), in the stage of full flowering and in very late system (A_4) in the stage of post-flowering. In a system with two pre-cuts (A_5), at the time of cutting, plants were in the budding stage and in final cut, plants were in various stages of intensive growth. Cutting of growth for forage was done manually at height of about 5 cm.The number of plants was determined by counting the number of plants per meter in all replications in the seed cut. The crop was established in 2002 and seed production started from the second year (2003). The number of plants was determined in the second, third (2004), fourth (2005), fifth (2006) and sixth year (2007).

Statistical processing of obtained data was done by variance analysis. Testing of the significance of differences was done by LSD test.

Results and Discussion

The number of plants (tab. 1) in the test period ranged from 205 (the second year of examination, treatment A_5) to 34 (the sixth year of treatment A_4). In the second year of life and the first year of exploitation of crop intended for seed production, the average number of plants was 199 plants m^{-2}, and there were no significant differences between treatments. In the present research, almost identical number of plants was obtained as in the results reported by Karagić (2004) 198.6 plants m^{-2} and Beković (2005) with sowing at 20 cm in the second year of study (197.3 plants m^{-2}). In the same research by Beković (2005) the variety K-28, the same inter-row distance, showed somewhat thinner structure (177.2 plants m^{-2}).

In the third year, it was registered that the number of alfalfa plants significantly decreases with age. The largest number of plants was recorded in the early harvest system (A_1) 153 plants per m^{-2}, and the lowest number of plants in the harvest system with two pre-cuts (A_5) 130 plants per m^{-2} or by 15.0% less compared to the harvest system A_1. For other systems of cutting the number of plants was uniform.

Tab.1 Impact of harvest system and the exploitation year on the number alfalfa plants m^{-2}

Year	A_1	A_2	A_3	A_4	A_5	Average
II	199	201	195	197	205	199
III	153	147	152	149	130*	146
IV	104	99	101	79*	84*	93
V	91	89	81	63**	66**	78
VI	60	58	55	34**	38**	49

* Significantly different P<0.05; ** significantly different P<0.01

In the third year, the average number of plants was 146 plants m^{-2} and it was similar to number of plants (141.9 plants m^{-2}) recorded in research by Beković (2005), and more than the number of plants (94 plants m^{-2}) reported by Karagić (2004).

The number of plants in the fourth year ranged from 79 to 104 and the average value of 93 plants m^{-2}. During the fifth year, the trend of reduction of the number of plants continued. The minimum number of plants was realized in the harvest systems A_4 and A_5 (63 and 66 plants/m^2, respectively), which was statistically significant difference compared to the other harvest periods. The number of plants decreased slower in the previous harvest systems and was the highest in the A_1 and A_2 systems.With the aging of crops, the number of crop plants decreased and the lowest was in the sixth year, average of 49 plants m^{-2}.

Considering the system of harvest, it can be concluded that the number of plants decreased from the first $A_1$to the last A_4harvest system. Particularly low was number of plants in the harvest systems A_4 and A_5 in relation to the number of plants obtained in the harvest systems A_1, A_2 and A_3.

Looking at the absolute decline in the number of plants we can see that in the third year the number of plants has declined by 53 plants m^{-2}, compared to the second year. A similar decline is recordedin the fifth relative to the fourth year, while in the fifth year the number of plants decreased by 15 plants m^{-2}, in the sixth it decreased by 29 plants per m^{-2}. It can be concluded that the year had a major impact on decline of the number of plants. In the first years of exploitation (for seed production), with denser crop structures, the decline in the number of plants was greater than in the later years of exploitation, which is confirmed by the results reported by many authors (Vučković, 1994;Beković, 2005).

The effect of the year on the number of plants per unit area can be explained as alfalfa tends to reduce the structure density with aging, but also growing conditions and harvest system during certain years had an impact on the number of plants.

In the third year in our research, the greatest decline in the number of plants was in the A_5 system (75 plants m^{-2}), which is in line with the results of Karagić (2004), in similar agro-ecological conditions in the harvest system with two pre-cuts, 20% less plants were recorded compared to the secondary system early harvest with one pre-cut. However, in the next year (fourth), the greatest decline occurred in A_4 system (70 plants m^{-2}) and A_3system(51 plants m^{-2}). Subsequently, in fifth year, the greatest decrease occurred in the A_5period of harvest (18 plants m^{-2}). In sixth year, a uniform reduction of the number of plants was recorded.

The greatest decline in the number of plants in the A_5 system of harvest in the third and fifth year was a result of more frequent cutting, i.e. cutting with two pre-cuts in the A_5 harvest system. In our research, the distance between the two harvests in the system A_5 was approximately 30 days, which is according to Undersander (2004), in terms of persistence of crops, short period.

Sheaffer et al. (1988) report that the storage and use of non-structural carbohydrates has a cyclical nature; from the decline in the early vegetative growth to a height of 20 to 30 cm,

after which the amount of carbohydrates increases with the development of plants to full bloom, but generally an adequate level is considered at the beginning of flowering.
O 'Rourke and Millar (1996),quoted by Krnjaja (2004) report that populations of *Fusarium spp*. in cases of frequent cutting, significantly increase in the rhizosphere compared to other types of fungi. The stresses in plants, such as the insufficient potassium fertilization, frequent cutting and disease, clearly affect the greater presence of *Fusarium spp*. on the alfalfaroot. Also, there is a causal link between the presence of *Fusarium spp*. and deterioration of the root crop.
The counting of the plants was carried out during the harvest of crops. In the second year, the same treatments were applied(frequent cutting in the two pre-cuts) as well as in subsequent years, but in that year there was no change in the number of plants. This indicates that the number of plants was also under the influence of autumn harvest. Seed harvest in the A_4 and A_5 system was in September, which is according to the opinion of Silkett et al. (1937)andRather and Harrison (1938) cit. Sheaffer et al. (1988), Undersander (2004), cutting in the,, critical period "in which alfalfahad not enough time to make up for reserves in the roots and thus enterthe winter period more prepared. Postponement of the first pre-cut harvest influenced also somewhat later seed crop harvest and subsequent regeneration which contributed to the reduction of yield in the autumn, confirming the lower level of development of crops with late cut. The lowest yield and crop development were recorded in the harvest system A_5. Later seed harvest contributed to the formation of lower amount of carbohydrates in the roots in the autumn, so plants, going from A_1 to A_5 system, entered winterwith lower reserves, which had adverse effect on the abundance of plants, especially prevalent in the A_5 system of harvest.
Considering the number of plants in the A_4 and A_5 systems it can be seen that in the second year the number of plants was even, and that in the third year of the A_5 system lower number of plants in relation to the A_4 (and other systems) was obtained. However, in the fourth year (2005) there was a significant decline in the number of plants in the system A_4, followed by the lowest number of plants in this system until the end of exploitation. It can also be noted that in the fourth year a significant decline in all variants occurred. Average decrease was 53 plants m^{-2} and almost identical decline which occurred in the previous year, while in the next year the average reduction of only 15 plants m^{-2} was recorded.
Undersander et al. (2004) report that excessive soil moisture in autumn interfered with acclimatization of plants and increased predisposition to winter damage**.** A major decline in the number of plants in the fourth year was likely due to the large amount of rainfall. From September 2004 to September 2005, there was 879 mm of rainfall.
Particularly pronounced was the precipitation in November 2004 and May and August 2005. During the experiment, in November 2004, even 141mm of rain was recorded, which probably contributed to plants with increased predisposition to winter damage to enter the winter period.
One of the most important pathogens that attack alfalfa and induce the shortening of life expectancy of alfalfa in Serbia is *Colletotrichum trifolii* (Vasićet. Al., 2005, Vasić2007).The major types of*Colletotrichum* attack legumes at a temperature of 18-20^{o}C and when the relative air humidity is above 90% (Lenn cit. Bailey and Jeger, 1992).The authors note that frequent grazing or cutting reduce spreading and development of inoculum and development of disease from the group of *Colletotrichum* genus.
On the other hand a greater degree of damage in winter increased the susceptibility to diseases in the subsequent year. In May 2005,precipitation amount was 104 mm, which probably favored the development of diseases of the *Colletotrichum* genus and led to diseases occurring in the late cut (A_4) when the temperature was higher, which is favourable to the development of these pathogens.

This, together with wet conditions in 2005, in addition to increased relative decline in the number of plants in relation to the previous year, resulted in lower reduction of the number of plants in variants of the earlier harvest (A_1, A_2, A_3), and in particular in the variant with two pre-cuts (A_5). Delaying first cutting in the extremely humid year 2005 probably led to the greater thinning of crops and as a result, the A_4 harvest system in 2005 realized the least number of plants.

Finally, it can be concluded that more frequent cutting and later harvesting seed in the fall, led to a greater decline in the number of plants in the A_5 harvest system. In addition, the above combination of factors, such as large amounts of rainfall during the year, especially in November and May and later first harvest have contributed to decline in the number of plants, especially noticeable in A_4 harvest systems.

Conclusions

Growing conditions during certain years and harvest system had effect on the number of plants. Number of plants declines with aging. The years of exploitation had a major impact on decline of the number of plants**.** In the first years of production, in densercrop structures and extreme climatic conditions, decline in the number of plants was greater than in the later years of exploitation and moderate conditions during the year. In a system with two harvest pre-cuts wherethe third cutwas intended for the seed production, at the beginning there was a rapid decline in the number of plants. Also, in years with high rainfall in November and May, and later first harvest,the decline in the number of plants was greater. The minimum number of plants was recorded in the production of seed,from the second harvest in the system of very late first harvest and in the system where the seed production was realized from the third growth.

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LONG-TERM EFFECTS OF INCORPORATION OF CROP RESIDUES AND INCREASING DOSES OF NITROGEN ON THE MAIZE YIELD

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Abstract

Study of the effects of crop residue incorporation (CRI) combined with fertilization by increasing nitrogen (N) doses on grain yield of two maize hybrids was carried out on a long-term stationary experimental field of the Institute for Field and Vegetable Crops, established in 1971 at Rimski Šančevi, Novi Sad, Republic of Serbia. The trial involved the following variants: a) treatments with CRI plus use of increasing nitrogen doses: 0 (Ø; control variant), 60, 90, 120, 150 and 180 kg of pure nitrogen per ha, and b) treatments without CRI and using of next increasing nitrogen doses: 0, 90 and 150 kg N ha^{-1}. Grain yield obtained by the treatment with CRI on average for all variants of N-fertilization was 8.38 tha^{-1}, being for 1 tha^{-1} higher in relation to the average yield on treatments without CRI. On average, the highest yield for the both of the studied hybrids (9.09 tha^{-1}) achieved by fertilization of 180 kg N ha^{-1} with CRI, and was statistically significantly higher compared to all variants in treatments without incorporation of crop residues. Positive effects of long-term CRI to maize grain yields in comparable N-variants, depending on hybrid and doze of the applied nitrogen varied in the range of 180 to 1530 kg ha^{-1}.

Key words: *maize, crop residues, nitrogen, grain yield.*

Introduction

Crop residues present the mass of organic matter left on the field after harvest or picking of the main products. They can be used in many different ways: as animal feed, litter in livestock production, for ploughing (incorporation in the soil), for mulching, composting and preparation of artificial manure, as a material for energy production etc. If the farm does not have livestock and crop residues cannot be used as animal feed or litter, and be returned to the soil improved as manure, incorporation of crop residues has advantages in comparison to the other ways of use. They represent significant quantity of bio-mass that has extremely important role in cycling of agricultural ecosystem matters, especially under condition of insufficient use of organic fertilizers.

Fertilization with organic and mineral fertilizers has positive effect to humus content. Organic plant nutrition returns organic matter directly into the soil and by use of mineral fertilizers, increases plant aerial and root mass. It is known that incorporation of organic fertilizers and crop residues does not only improve nutritional land potential, but also water capacity, aerial and thermal soil properties as well as microbiological activity necessary for mobility of biogenic elements (*Čuvardić, 1993*).

In our fields, crop residues are often burned, causing partial or complete loss of biogenic elements. Burning of crop residues is undesirable practice prohibited by our legislation. In a long term, permanent removal of plant residues from fields or their burning is very harmful, which is reflected in nitrogen and humus loss, and destruction of living world in it. Crop residues are characterized by a relatively high content of biogenic elements essential for plant nutrition that are completely or partially lost by burning (*Latković et al., 2009*). For the purpose of organic matter increase in soil, it is necessary to increase incorporation of biomass

and crop residues by ploughing, or to apply organic fertilizers. Fate of crop residues (ploughed, burned or removed) has significant effect to the amount of nutrients that are need to be applied.

Incorporation of plant crop residues has positive effect to soil quality, primarily on organic matter content. By ploughing, plant residues are included in the process of organic matter cycling in soil, improves biological activity and structure of soil as well as water-aerial and thermal soil properties. Incorporation of hay or maize stalks into soil cannot increase the amount of humus suddenly nor rapidly – it is slow and a time consuming process, but it can improve soil structure, which is especially important for heavy soils. This enables better air and water regime, better moisture uptake and keeping, forming of the favourable structure and "biological maturity" of soil, which allows easier and better late soil cultivation, with low fuel consumption *(Malinović and Meši, 2008).*

In plant production mass of crop residues (hay, stems, leaves, and roots) can be rather large. For instance, in maize it is even up to 10 tha^{-1}, in wheat 4-6, in sunflower 4-5, in soybean about 4 tha^{-1}, and in sugar beet even 40-60 tha^{-1}. Ploughing, but not removal or burning of this mass, with combined use of organic and mineral fertilizers has effect on significant increase in nutrient content and availability, increase humus content in soil, and thus its general fertility. Application of mineral fertilizers only can partially and in short term amortize harmful effect of organic matter removal and burning of crop residues. It was established that last in last decades humus content in Vojvodina soil decreased for 0.2 to 0.81%, on average for 0.38% *(Bogdanović et al., 1993).* In trials carried out in our country and in the world, convenient effect of incorporation of crop residues to yield and its quality has been proved on increase of total nitrogen and carbon, improved soil fertility or reduction in nitrogen leaching *(Nicholson et al., 1997; Powlson et al., 1987),* and on grain yield increase *(Pracházková et al, 2002; Silgram et al., 2002; Latković et al., 2012).*

The aim of the research in this paper was to examine impact of effect of crop residues incorporation in combination with different nitrogen doses on grain yield of two maize hybrids. It was expected that the obtained results justify long-term ploughing of crop residues providing higher grain yield.

Materials and methods

Two years lasting study of the effect of crop residues incorporation (CRI) with different nitrogen fertilization doses on the yield of two maize hybrids was performed on international long-term stationary field trial (called ISDV - *Internationale Stickstoff Dauer Versuche*). The experiment began in 1971, within the series of trials of the International Commission for the study of soil fertility, at the experimental field of the Institute for Field and Vegetable crops in Rimski Šančevi, Novi Sad (Serbia). The soil on which the experiment was performed is the calcareous chernozem on loess, with slightly alkaline reaction, about 3% of humus in the upper layer, medium supplied with easily available phosphorus and well supplied with potassium. Maize is grown in three-crop rotation system (maize-soybean-wheat). Maize was planted at a row spacing of 75 cm, and the distance between plants in a row was 25 cm. The row length per plot was 7 m. Preceding crop for maize was wheat. Maize sowing was performed in optimal sowing time for conditions in Vojvodina - in mid-April in 2012 and 2013. The study presents the average results of these two years of the experiment. The trial was two factorial in randomized block design and carried out in 4 replications. The trial had nine variants, of which six were with ploughing of crop residues (CRI), and three without it. The experiment involved the following variants of nitrogen fertilization (Factor A):

a) Treatments with CRI and use of increasing nitrogen doses: 0 (Ø; control variant), 60, 90, 120, 150 and 180 kg of N ha^{-1} (each third year under maize 5 tha^{-1} of crop residues were

ploughed, with addition of 50 kg of N ha^{-1} from the mineral fertilizer (10 kg N per 1t of CR) in order to prevent nitrogen depression),
b) Treatments without CRI and with application of increasing nitrogen doses of: 0, 90 and 150 kg of pure N ha^{-1}.
As objects of investigation two maize hybrids were selected (Factor B): NS-540 and NS-6030. Application of the envisaged nitrogen doses in the trial was conducted in two occasions: ½ in autumn, before the basic tillage and ½ in spring (pre-sowing treatment of maize). In all variants, constantly the same amount of phosphorus and potassium were applied: 80 kg of P_2O_5 and K_2O ha^{-1} in autumn before the basic tillage.
The obtained results of maize grain yield in different trial treatments with and without CRI and with different nitrogen supply were statistically processed by the method of analysis of variance for two factorial *split-plot* design (statistical software GenStat v.9.1.) where statistically significant differences of treatments was tested by LSD test.

Results and discussion

Based upon variance analysis of maize grain yield in the trial (Table 1), significant impact on yield on the bases of F-test showed fertilization system (F_{pr}=0.013*) and interaction of fertilization and hybrids (F_{pr}=0.026*), while hybrids did not have more significant impact to grain yield (F_{pr}=0.184). Observation of the percentage share of individual sources of variation in the sum of total squares, indicates also that fertilization variants had the largest share in the total of yield variation (28%), followed by interaction A*B (15%), while the hybrid participated in yield variation with only 1%.

Table 1. Analysis of variance of maize grain yield

Sources of variation	Degrees of freedom	Sum of squares	Sum of squares (%)	Mean of squares	F-test	Probability of F-test (F_{pr})
Replications	3	19.137	10	6.379	3.32	/
Fertilization variants (A)	9	51.991	28	5.777	3.00	0.013*
Error	27	51.904	28	1.922	1.61	/
Hybrid (B)	1	2.205	1	2.205	1.85	0.184^{ns}
Fertilization*hybrid (A*B)	9	27.446	15	3.050	2.55	0.026*
Error	30	35.836	19	1.195	/	/
Total:	79	188.519	100	/	/	/

The achieved average grain yield for the trial in whole was 8.05 tha^{-1} (Table 2). However, grain yield obtained by the treatment with ploughing of crop residues (average for all variants of nitrogen fertilization) was 8.38 tha^{-1}, and it was for 1 tha^{-1} (i.e. for 13.55%) higher in comparison to the average for fertilization variants without ploughing of crop residues (7.38 tha^{-1}).
For the both of the studied hybrids, the highest average yield of 9.09 tha^{-1} was obtained by fertilization with 180 $kgha^{-1}$ of nitrogen with ploughing of crop residues. However, grain yield obtained in this variant was statistically significantly higher only in relation to variants in the treatment without ploughing of crop residues. The other differences between fertilization variants were not statistically significant.

Table 2. Grain yield (tha^{-1}) with different N doses in treatments with and without incorporation of crop residues

Fertilization variant (A)			**Hybrid (B):**		**Average (A):**
	No.	**Doses of N (kg ha^{-1})**	**NS-540**	**NS-6030**	
With incorporation of crop residues	1	**0**	8.24	7.51	**7.88**
	2	**60**	9.30	8.07	**8.69**
	3	**90**	8.60	7.46	**8.03**
	4	**120**	8.65	7.69	**8.17**
	5	**150**	7.85	9.06	**8.46**
	6	**180**	8.86	9.32	**9.09**
	Average:		**8.58**	**8.19**	**8.38**
Without incorporation of crop residues	7	**0**	7.23	7.33	**7.28**
	8	**90**	7.07	7.56	**7.32**
	9	**150**	6.96	8.12	**7.54**
	Average:		**7.09**	**7.67**	**7.38**
Average (B):			**8.08**	**8.01**	**8.05**
		A	B	B*A	A*B
LSD	5%	1.42	0.50	1.77	1.58
	1%	1.92	0.67	2.36	2.13

In the treatment without ploughing of crop residues, the highest grain yield of 7.54 tha^{-1} was achieved with the highest dose of the fertilizer - 150 kg N ha^{-1}, however, there were no statistically significant differences in comparison to the control and variant with 90 kg N ha^{-1}. Results of *Starčević et al. (1999)* indicate statistical and economic justification of maize fertilization by nitrogen in doses that do not exceed 120 kg ha^{-1}, which is partially in agreement with the obtained results in this work.

On average for all fertilization variants, hybrid NS 540 (8.08 tha^{-1}) did not achieve statistically significantly higher yield in comparison with hybrid NS 6030 (8.01 tha^{-1}), i.e. the yield was higher for only 70 kg ha^{-1}. On the treatment with ploughing of crop residues, this difference between hybrids was slightly higher for hybrid NS 540 (for 390 kgha^{-1}), but it was also statistically insignificant. However, in the treatment without ploughing of CR, hybrid NS 6030 (with grain yield of 7.67 tha^{-1}) achieved yields that was for 580 kgha^{-1} significantly higher in comparison to hybrid NS 540 (7.09 tha^{-1}).

Hybrid NS 540 had the highest yield of 9.30 tha^{-1} achieved by doses of 60 kg N ha^{-1} on treatment with crop residues ploughing. In this variant, the yield was significantly higher in comparison with all three doses of N on treatments without crop residues ploughing, but in relation to other doses of N on treatment with crop residues ploughing statistically significant differences were not found. Fertilization with 180 kg N ha^{-1} and with crop residues ploughing also resulted with statistically significantly higher yield compared to variants with 90 and 150 kg N ha^{-1} on treatment without crop residues ploughing.

Hybrid NS 6030 had the highest grain yield of 9.32 tha^{-1} achieved by fertilization with the highest N doses with crop residues ploughing. In this variant, grain yield was statistically significantly higher compared to the control and variant with 90 kg N ha^{-1} on treatment with crop residues ploughing, as well as in relation to the control on treatment without crop residues ploughing.

Positive effect of long-term crop residues ploughing on maize grain yield in comparable trial variants is presented in Table 3, and depending on studied hybrids and doses of the applied nitrogen, it was within limits of only 180 kg of grains for hybrid NS 6030 in variant without nitrogen fertilization, up to 1530 kg ha^{-1} for hybrid NS 540 fertilized with 90 kg N ha^{-1}.

Table 3. Effect of CRI to maize grain yield (tha^{-1})

Nitrogen rate	Crop residue (CR)	Hybrid (B)		Average
		NS-540	NS-6030	
0 kg N ha^{-1}	With CR incorporation	8.24	7.51	**7.88**
	Without CR incorporation	7.23	7.33	**7.28**
	Difference:	**1.01**	**0.18**	**0.60**
90 kg N ha^{-1}	With CR incorporation	8.60	7.46	**8.03**
	Without CR incorporation	7.07	7.56	**7.32**
	Difference:	**1.53**	**-0.10**	**0.72**
150 kg N ha^{-1}	With CR incorporation	7.85	9.06	**8.46**
	Without CR incorporation	6.96	8.12	**7.54**
	Difference:	**0.89**	**0.94**	**0.92**
Average for all 3 N doses:	With CR incorporation	**8.23**	**8.01**	**8.12**
	Without CR incorporation	**7.09**	**7.67**	**7.38**
	Difference:	**1.14**	**0.34**	**0.74**

On average for both hybrids, in control variant without nitrogen use, CRI increased grain yield for 600 kgha^{-1}; in variant with 90 kg N for 720 kgha^{-1}, while the most intensive fertilized variant with 150 kg N ha^{-1} and CRI provided the highest effect in grain yield increase - 920 kgha^{-1}.

On average for both hybrids and all three nitrogen doses, CRI increased yield for 740 kgha^{-1}; hybrid NS 540 had increase of 1140 kg, i.e. 340 kgha^{-1} for hybrid NS 6030. Table 3 shows that hybrid NS 540 in control and with use of 90 kg N ha^{-1} had significantly better reaction to CR ploughing, while the use of the highest N dose was reverse, i.e. hybrid NS 6030 gave a higher yield in comparison to NS 540.

In literature there many examples in which CRI provides identical results as well as manure application in soil properties improvement and yield height. Use of N fertilizers also significantly increases quantity of plant residues which incorporation in to the soil increases humus content and efficiency of carbon retention in the soil (*Halvorson et al., 1999*). In trials carried out in our country and in the world, beneficial effect of CR ploughing to yield and its quality has been proved (*Latković et al., 2011; 2012*).

Conclusion

Based upon results of the study on affect of growing nitrogen doses to maize grain yield in variants with or without CR ploughing, the following conclusions can be made:

Grain yield obtained in treatment with CR ploughing, on average for all nitrogen fertilization variants was 8.38 tha^{-1}, being for 1 tha^{-1} higher compared to the average in variants without CR ploughing.

On average, the highest yield for the both of the studied hybrids obtained with 180 kg N ha^{-1} with CR ploughing and was statistically significantly higher compared to variants in treatment without CR ploughing.

Positive effects of long-term CR incorporation to maize grain yields in comparable N-variants, depending on hybrid and doze of the applied nitrogen varied in the range of 180 to 1530 kg ha^{-1}.

On average for both hybrids, in control without nitrogen use, CR ploughing increased grain yield for 600 kg ha^{-1}, in variant with 90 kg N for 720 kg, while in variant with the most intensive fertilization, CR ploughing resulted in grain increase for 920 kg ha^{-1}.

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MINERAL NUTRITION USE EFFICIENCY OF WINTER WHEAT DEPENDING ON THE INTENSITY OF FERTILIZATION

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Abstract

Research on efficiency of mineral nutrition of winter wheat in three production years were carried out in long-term field experiment at the Institute of Field and Vegetable Crops at Rimski Šančevi location, Novi Sad, Serbia. This paper presents the results about effects of mineral nutrition on grain yield of winter wheat in 20 different fertilization treatments with increasing rates of nitrogen, phosphorus and potassium, and agronomical use efficiency of the applied fertilizers. As expected, nitrogen had significantly highest impact on the wheat yield. The highest increase in the grain yield with one kilogram of the fertilizer used was with nitrogen (33.89 kg of grain/1 kg of N, on average for three years), followed by phosphorus (9.11 kg of grain/1 kg of P_2O_5), and potassium (3.01 kg grain/1 kg of K_2O). Agronomical N use efficiency differed depending upon weather conditions of the year, but in all analyzed years as well as on average, it had tendency to decrease if intensity of fertilization increased.

Keywords: *wheat, yield, fertilization, nitrogen, nutrient efficiency*

Introduction

It is well known that deficiency of mineral nutrients, as well as high fertilizer rates can cause reduction in wheat yield. Use of higher amounts of fertilizers than optimal provides not only economically inadequate results from the aspect of direct investments, but such rates can be also harmful for a large number of plant species and varieties (lodging and more intensive occurrence of diseases, etc.), and are a common cause of agroecosystems pollution.

Efficient nitrogen fertilization is one of the key elements for economical production of wheat, but also for protection of underground and above ground waters from pollution caused by leaching of nitrates due to excessive and inappropriate application of N *(Vuković et al., 2008)*. Efficiency of nitrogen application in winter wheat (WW) is valuable indicator of rational N-fertilization. The term "*Nitrogen Use Efficiency*" *(NUE)* has several definitions and calculation procedures, depending on the purpose of the study *(Dobermann, 2005).* In agricultural practice the most wide spread is use of *Agronomic N use Efficiency (AE_N),* based on the "method of difference" and it is determined as the ratio of the yield increase achieved by N-fertilization and used amounts of N *(Craswell and Godwin, 1984; Dobermann, 2005)*: $AE_N=\Delta GY/F_N$ *(kg grain / 1 kg N)*; where ΔGY = grain yield on the plot fertilized by nitrogen – grain yield on control plot (without N use); F_N – amount of applied N in fertilized treatment (plot). *Dobermann (2005)* states that typical values of AE_N in wheat are between 10–30 kg of grain per 1 kg of the applied N, and values >30 kg kg^{-1} are found in well organized growing systems or at low levels of nitrogen fertilization and on poor soils. *Raun and Gordon (1999)* stated that on the global level, worldwide NUE in cereal production is 33%.

Materials and methods

Research on WW mineral nutrition efficiency was conducted in a long-term stationary field trial, established in 1965 at the experimental fields of the Institute of Field and Vegetable Crops at Rimski Šančevi location, Novi Sad, Serbia. The trial is based on 4-year crop rotation

(four fields) including sugar beet, maize, sunflower and wheat. In this paper results from three years of experiment (2010/11-2012/13) are presented.

The trial was set up on a calcareous chernozem soil type, with slightly alkaline reaction (pH = 7.64), moderate content of humus (3.27%) and readily available phosphorus (15.98 mg/100 g of soil) and with high content of readily available potassium (25.64 mg/100 g of soil). The experiment was set up in 4 separate fields, where different crops were grown on different fields each year. The size of each field is 68 x 270 m and is divided into 4 replication with 20 experimental plots in every replication, meaning that every field is divided into 80 plots with randomized treatments (different NPK doses and ratios), where every plot is additionally divided into more subplots, depending on the number of varieties examined. Fertilization treatments were:

1. Control (unfertilized plot)
2. N_2
3. P_2
4. K_2
5. N_2P_2
6. N_2K_2
7. P_2K_2
8. $N_1P_1K_1$
9. $N_1P_2K_1$
10. $N_1P_2K_2$
11. $N_2P_1K_1$
12. $N_2P_2K_1$
13. $N_2P_2K_2$
14. $N_2P_3K_1$
15. $N_2P_3K_3$
16. $N_3P_1K_1$
17. $N_3P_2K_1$
18. $N_3P_2K_2$
19. $N_3P_3K_2$
20. $N_3P_3K_3$

where index number represents doses of pure active matter of each nutrient: $_1$=50, $_2$=100, $_3$=150 kg of N, P_2O_5 and K_2O per ha.

Each year standard WW cultivation practice for agro-ecological conditions in Vojvodina province was applied. The whole amount of P_2O_5 and K_2O and the half of the N dose were applied in autumn before tillage. Remaining amount of N fertilizers for WW was given in spring at topdressing and before seeding for other species in the trial. Wheat straw and harvest residues of other crops are ploughed under after harvest. In all examined years, sowing was done in optimal sowing time for conditions of Vojvodina (in October) with sowing density of 500 viable seeds per m^2, and spacing between rows of 12.5 cm. Application of pesticides was done sporadically, only if it was necessary. For investigation purpose, WW variety Zvezdana was chosen, as one of the most widespread in Vojvodina region.

Weather conditions (table 1) in 2010/11 and 2011/12 indicates that both years were moderately dry, mostly due to low precipitation amounts in March, i.e. at the beginning of spring vegetation, but also in June, during the period of grain filling and ripening. In comparison to Long-Term Average values (LTA for period 1964-2012), in 2010/11 growing season there was only 389 mm of rainfall, i.e. for 63 mm less than LTA (452 mm).

Table 1: Precipitation and temperature conditions at Rimski Šančevi experimental station (N 45° 19', E 19° 50') during WW vegetation period (X-VI)

Year		Month									WW veget. period (X-VI)	Difference to LTA
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.		
T (°C)	**2010/2011**	9.1	9.5	0.8	0.1	-0.2	6.0	13.2	16.8	20.9	**8.5**	**0.2**
	2011/2012	10.7	2.8	4.2	2.0	-5.0	8.0	13.0	17.2	22.5	**8.4**	**0.1**
	2012/2013	13.6	10.0	1.0	3.1	5.8	6.0	13.4	18.1	20.4	**10.2**	**1.9**
	LTA[1]	**11.5**	**6.1**	**1.4**	**-0.4**	**1.5**	**6.3**	**11.4**	**16.9**	**20.0**	**8.3**	**-**
P (mm)	**2010/2011**	67	47	64	25	37	26	23	63	37	**389**	**-63**
	2011/2012	35	2	49	43	67	4	83	51	31	**365**	**-87**
	2012/2013	49	36	55	60	1	68	30	118	126	**543**	**91**
	LTA[1]	**47**	**50**	**50**	**39**	**34**	**38**	**48**	**60**	**87**	**452**	**-**

[1]LTA – Long-Term Averages (1964-2012)

In 2011/12 during WW growing season in relation to LTA, precipitations were for 87 mm lower. Average temperature conditions in these years were on the LTA level, with higher deviations in November and December, but probably without negative effects on WW. However, temperature conditions in April, period of intensive growth (stem elongation), were

slightly warmer (about 2 ºC), which in addition with dry Mart and June in 2012 could decreased WW yield in this year. 2012/13 production year was characterized by more favorable precipitation and temperature conditions during the spring part of growing season and earlier start of the vegetation period, accompanied with heavy rainfall in May and June, resulted in a significant increase in grain yield.

Results and discussion

Results in table 2 shows average WW yields and its variation to different amounts applied and ratios of NPK nutrients in 20 different fertilizing treatments in 3 analyzed years. Average WW yield in the trial was 5.68 t ha^{-1}, ranging from 2.86 to 6.94 t ha^{-1}. Average mean yield deviation per years and fertilizing treatments from the overall mean was ±1.41 t ha^{-1} i.e. average mean relative yield deviation (CV) was 25%.

The effects of fertilization on WW yield were significant in all years. Significantly lower yields in comparison to all other treatments were obtained on treatments without nutrient application (control treatment), followed by the treatments with only potassium applied (K_2 treatment), only phosphorus (P_2) and on treatment P_2K_2 (table 2).

Table 2: The effect of mineral nutrition on wheat grain yield (t ha^{-1})

Mineral nutrition treatment	kg ha^{-1} N	P_2O_5	K_2O	Years 2011	2012	2013	Average 2011-2013
Ø	0	0	0	1.71	1.80	5.07	**2.86**
N_2	100	0	0	4.40	5.73	7.07	**5.73**
P_2	0	100	0	2.25	1.42	6.05	**3.24**
K_2	0	0	100	2.01	1.82	5.31	**3.04**
N_2P_2	100	100	0	5.31	6.44	7.35	**6.36**
N_2K_2	100	0	100	4.76	5.49	7.01	**5.75**
P_2K_2	0	100	100	2.67	2.08	5.08	**3.28**
$N_1P_1K_1$	50	50	50	3.73	4.83	7.21	**5.26**
$N_1P_2K_1$	50	100	50	4.48	4.80	7.69	**5.65**
$N_1P_2K_2$	50	100	100	4.43	4.52	7.37	**5.44**
$N_2P_1K_1$	100	50	50	5.18	5.76	7.80	**6.25**
$N_2P_2K_1$	100	100	50	5.78	6.25	7.77	**6.60**
$N_2P_2K_2$	100	100	100	6.13	6.23	7.64	**6.67**
$N_2P_3K_1$	100	150	50	6.03	6.44	7.58	**6.68**
$N_2P_3K_3$	100	150	150	6.38	6.39	7.92	**6.90**
$N_3P_1K_1$	150	50	50	6.18	6.32	7.27	**6.59**
$N_3P_2K_1$	150	100	50	6.39	6.45	7.42	**6.75**
$N_3P_2K_2$	150	100	100	6.31	6.76	7.25	**6.77**
$N_3P_3K_2$	150	150	100	6.80	6.63	7.40	**6.94**
$N_3P_3K_3$	150	150	150	6.55	6.52	7.15	**6.74**
Average:				**4.87**	**5.13**	**7.02**	**5.68**
Max				**6.80**	**6.76**	**7.92**	**6.94**
Min				**1.71**	**1.42**	**5.07**	**2.86**
Max-Min				**5.08**	**5.34**	**2.86**	**4.08**
SD				**1.63**	**1.84**	**0.90**	**1.41**
CV (%)				**34**	**36**	**13**	**25**
LSD		$_{0,05}$		0.62	0.60	0.71	0.58
		$_{0.01}$		0.86	0.84	1.01	0.73
r		N		0.94*	0.93*	0.72*	0.92*
		P_2O_5		0.59*	0.43*	0.45*	0.51*
		K_2O		0.39	0.25	0.16	0.29

*significant on the level α=0.05

Considering the effect of a *single nutrient application*, it can be concluded that N had the most significant influence on the WW yield. In average for all investigated years, by applying nitrogen only (N_2 treatment) yield increased for 2.88 t ha^{-1} (101%) in comparison to control. On treatment with only phosphorus applied (P_2), yield increased for 380 kg ha^{-1}, i.e. for 13%, while the potassium didn't have any significant effect on the yield. When these three elements were used in *dual combinations*, it can be observed that fertilization with N and P (N_2P_2) had

the significant advantage in relation to combined application of N and K (N_2K_2). However, both treatments were significantly better than the treatment P_2K_2.

The highest WW yield in the trial (6.94 t ha^{-1}) was obtained on the treatment $N_3P_3K_2$, but statistically equally high yields (α=0.05; i.e. over 6.36 t ha^{-1}) were obtained on other triple fertilizing variants with the highest or moderate N doses, regardless to amounts of P and especially K. By applying all three nutrients at moderate or high N amounts (all treatments with $\mathbf{N_2}P_xK_x$ and $\mathbf{N_3}P_xK_x$; except $N_2P_1K_1$), yield ranged from 6.59 to 6.94 t ha^{-1}, i.e. varied only 350 kg. Statistical analysis didn't show any significant differences between these treatments. Higher doses of nutrients in these cases were not economically justified.

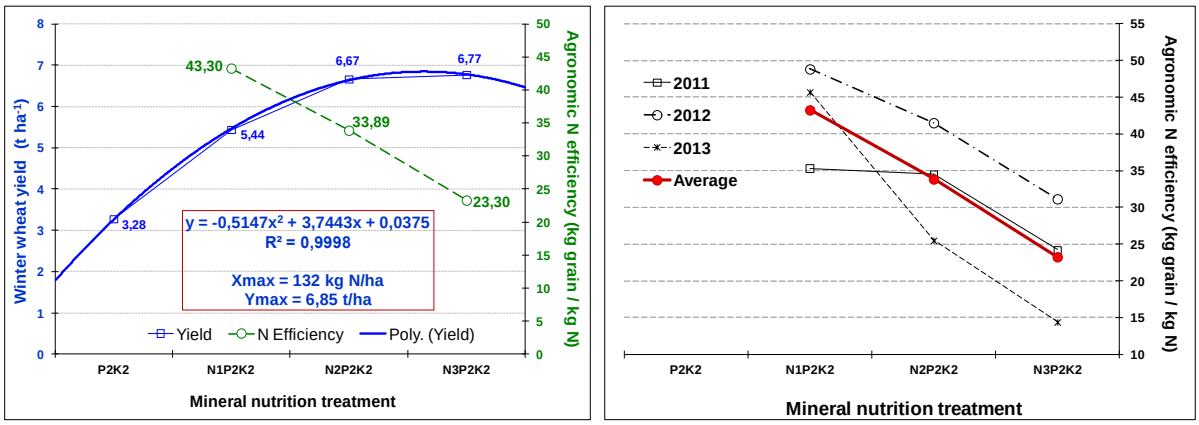
In order to get more complete insight into the statistical significance of individual nutrients in WW mineral nutrition, simple coefficients of correlations between applied amounts of nutrients and WW yields were calculated (table 2). In all analyzed years, as well as in three years average, there was a high and statistically significant (α=0.05) correlative relationship between N application and yield (r=0.72-0.94). Significant moderate correlations (0.43-0.59) was noticed between P application and yield, while correlation between applied amounts of K and yield was found no significant ($r=0.16-0.39^{ns}$).

Nutrient efficiency in WW yield formation: Complete influence of certain nutrients on plants (nutrient efficiency) is not easy to determine because of their different effects with individual and combined application. According to *Sarić and Jocić (1993)*, with combined application of nutrients, interaction among these nutrients occurs. For this reason, single nutrient effect can't be easily and precisely determined. However, by using "difference method", i.e. by comparing yield obtained at the joint application of NPK and treatment where the one of the nutrient is omitted, approximate contribution of a missing element to yield formation could be determined. In this specific case, to determine the effect of each single nutrient to grain yield formation, method of difference between the yield achieved on treatment with balanced mineral nutrition ($N_2P_2K_2$) and dual combinations where one of the nutrient is omitted (N_2P_2, N_2K_2 and P_2K_2) was used. From the data presented in table 3, it can be noticed that the N had the highest influence on yield increase per 1 kg of nutrient applied (33.89 kg of grain/1 kg of N, on average for three years), followed with the P (9.11 kg grain/1 kg P_2O_5) and K (3.01 kg grain/1 kg K_2O). Low values obtained with K-application indicate good supply of this nutrient in soil.

Table 3: Grain yield increasing with 1 kg of nutrients applied

Years	Grain Yield (t ha^{-1})				Yield increasing with 1 kg a.m. of nutrients applied (kg grains / 1 kg nutrient)		
	$N_2P_2K_2$	**P_2K_2**	**N_2K_2**	**N_2P_2**	**N**	**P_2O_5**	**K_2O**
2011	6.13	2.67	4.76	5.31	34.58	13.62	8.17
2012	6.23	2.08	5.49	6.44	41.55	7.46	-2.03
2013	7.64	5.08	7.01	7.35	25.54	6.26	2.88
Average	**6.67**	**3.28**	**5.75**	**6.36**	**33.89**	**9.11**	**3.01**

Influence of nitrogen fertilization doses on its efficiency. One of the main aims of this paper was to determine how different N fertilization levels influenced WW grain yield and nitrogen use efficiency (NUE). Determination of *„Agronomic N use Efficiency"*- AE_N (in this case effect of fertilization with increasing N doses on its efficiency) was done by the difference method, between yields on treatment $\mathbf{P_2K_2}$ (taken as control) and $\mathbf{N_1}P_2K_2$, $\mathbf{N_2}P_2K_2$ and $\mathbf{N_3}P_2K_2$. As can be seen from graph 1, average yield increase with 1 kg of N applied was the highest on treatment $\mathbf{N_1}P_2K_2$ (43.30 kg grain/1 kg N), then on treatment with moderate (33.89 kg/kg N) and the lowest on treatment with the highest N dose ($\mathbf{N_3}P_2K_2$; 23.30 kg grain/1 kg N).



Graph 1: The effects of increasing amounts of N on grain yield and yield increasing with 1 kg of nitrogen applied - agronomic N use efficiency, average results (left) and for all three years (right)

Agronomic N use efficiency differed among analysed years (graph 1, *right*). Highest AE_N values were in 2011/12 year and lower than average values in 2012/13. Differences could be partly explained by the climate conditions. In 2011/12 moisture reserve from February influenced intensive plant sorption of N applied at topdressing. Although March was dry, most N was already absorbed and more intensive absorption continued in wet April and May. In difference from this year, in extremely wet 2012/13 it is probably that the losses from N leaching were higher due to its removal to deeper soli layers.

In a specific year, as well as on average for all three years, AE_N had a decreasing tendency with increasement of N doses. The highest AE_N was on treatment with 50 kg N ha^{-1}, so this dose could be considered as the most rational in terms of lowest N losses and possible environmental pollution. However, from the aspect of joint influence on the yield of WW and nitrogen use efficiency, especially rational treatment was the one with 100 kg ha^{-1} of N applied (i.e. variant $N_2P_2K_2$).

With moderate amounts of P and K (treatments $N_xP_2K_2$; graph 1), the lowest N dose ($\mathbf{N_1}P_2K_2$) increased grain yield for 2.16 t in relation to treatment P_2K_2, which was taken as control. Next N dose increased yield for additional 1.23 t of grain, while the highest N dose (treatment $\mathbf{N_3}P_2K_2$) influenced non-significant yield increase of only 100 kg ha^{-1}. Average yield increase for every additional dose of 50 kg N ha^{-1} was 1.17 t of grain ha^{-1}. However, the first N dose influenced the most intensive yield increase compared to control, while the effect of following N doses was less expressed. Influence of increasing N doses on WW yield had the saturating effect, i.e. followed the shape of quadratic regression curve (R^2=0.99). Based on the equation of this regression, at moderate P and K doses (100 kg ha^{-1}), theoretically calculated maximum regression grain yield of 6.85 t ha^{-1} could be achieved by applying 132 kg N ha^{-1} (graph 1).

By analyzing the yield of WW during the 50 years trial period, *Kunzova and Hejcman (2009)* stated that in the fifth decade of the experiment, average yield increase per 1 kg of N applied was 18.7 kg of grain. Similarly to our results, *Vuković et al. (2008)* for conditions of Croatia found that NUE values decreasing with higher intensity of N fertilization. NUE values ranged from 9.21 kg kg^{-1} with 300 kg N ha^{-1} up to 24.13 kg kg^{-1} on treatment with 100 kg N ha^{-1}. Authors concluded that better N efficiency was at fertilization with 100 kg N ha^{-1}, which can be considered as rational amount in terms of grain yield and harmful influence on environment. Also, NUE was influenced by soli type and climatic conditions, mainly by precipitation and temperature regime during vegetation period *(Vuković et al., 2008)*. According to *Hatfield and Prueger (2004)*, NUE depended on soil moisture and N availability during vegetation. As well as in our research, *Pepó (2007)* concluded that the efficiency of fertilization was strongly modified by climatic conditions. *Ortiz-Monasterio et al. (2001), Ortiz-Monasterio (2002)* and *Raun and Gordon (1999)* stated that improvement in nutrition use efficiency by wheat can be achieved by two main strategies: application of more efficient

cultivation practices (amount and time of fertilizers application, nutrient source, etc.) and by breeding of varieties with better efficiency of nutrients applied.

Conclusion

In a 3-year investigation period, N had significantly the highest influence on the wheat grain yield. Fertilization with N only increase the yield compared to control up to 100%, while fertilization with K only wasn't statistically significant.

When nutrients were used in dual combinations, fertilization with N and P had the significant advantage in relation to combined application of N and K. However, both treatments were significantly better then the treatment PK.

The highest grain yield was obtained on treatment $N_3P_3K_2$, but statistically equally high yields were achieved on all other triple combinations with highest or moderate N doses.

The highest yield increase with 1 kg of nutrient applied was with N, then P, and lowest with K. Agronomic N use efficiency had the decreasing tendency with increasing N doses. In terms of joint influence on the yield of wheat and nitrogen use efficiency, especially rational treatment was the one with 100 kg of N ha^{-1} applied.

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INFLUENCE OF ORGANIC AND CONVENTIONAL METHODS OF GROWING ON QUALITATIVE PROPERTIES OF SOYBEAN

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Abstract

Two-year survey was conducted according to the principles of organic and conventional dryland cropping technologies. Experiment was placed in Backa Topola, on calcareous chernozem with wheat as preceding crop. In ecological production basic soil fertilization was performed with 15 $t \cdot ha^{-1}$ cowshed manure, and in conventional production as pre-sowing treatment was applied 100 kg $N \cdot ha^{-1}$. In both years were similar weather conditions. Examination factors were production ways and application of microbial fertilizer. Microbial fertilizer was in liquid state and it contained various types of microorganisms. Microbiological fertilizer used in both varieties of production of sample plots in five variations: 1-control (with out specific microbiological fertilizer), 2-treat fields seven days before planting, 3-during phenological stage 1-3 leaves, 4-first flovering, 5- lots of flovering. Data were processed by two-factorial split-plot experiment variance analysis method, and differences between treatments were analyzed by LSD-test. Correlation analysis was conducted. The aim of this work was to determine protein content and oil of organic and conventional cropping technologies and correlational dependency between surveyed characteristics. Average content of protein is 39.93% in 2012 was above that figure. Analyis effected plant breeding didn't recognised regularity of examined traits. Effect of application of microbiological fertilizer on protein and oil was very significant. Average oil content was higher in the organic and by 2.32% in comparison to the conventional production. Ecological soybean production, as production system with respect of environmental principles and standards, as well as specific local agroecological conditions, has its priority.

Keywords: *conventional and organic production, protein and oil content, microbial fertilizers, soybean*

Introduction

Areas that are seeded with soya variety, partially because achieved yields and also economic conditions but it has become an important factor crop production (Dozet, 2009). Soya grain is used in the form of various to process for human consumption. Therefore it's essential for part of soya production without applying mineral fertilizers and pesticides. Soya has important economic importance, mainly due to chemical structure of grain which has the following components around 40 % proteins and 20% oil. It's used in food industry as well as in different branch industries. Soya represents "popular products" on the global market. Retailing soya is of great importance practically in processing soya as flour, soya oil etc. Therefore it is especially achieved significant cooperation between primary agricultural production of soybeans and industry. (Cvijanovic D. and Cvijanovic G., 1989). Primary microbiological fertilizers are very important factor in the manufacture of soybeans which significant impact on its growth, envelopment and productivity (Cvijanovic et al., 2013). Soybean because of high content of proteins has very high nitrogen requirements, but as leguminous plants biggest part of nitrogen provides by nitrification. Instability of soya beans

very often represents a problem in industry animal feed, because of level of proteins and oils in biggest measure depend of genotype and environmental (Westgate et al., 2000). In agriculture production one of rate targets and guidelines is integrated and organic production, this means finding alternative ways of fertilization in order to avoid the consequences of land degradation (Cvijanovic et al., 2010). The prices of organic products still are higher than average from 15% to 30% in compared with products obtained commercially producing (Dozet et al., 2013).

Target of this work was to determine the protein and oil content in organic and commercial way of cultivation and with it determine the optimal variant of application effective microorganisms in both technology cultivation which will enable getting high, stable yield good chemical composition of soybeans with rational use of nitrogen and fertilizers. I addition, paper aimed at determining correlation dependency between important surveyed feature.

Materials and methods

Field research to examine the impact of soybean growing technology and application of microbiological fertilizers on quality of soybeans and other important features was carried out on the basis of set of plot in localities Backa Topola (Serbia), on carbonate chernozem, after wheat as a previous crop during 2012 and 2013. Plot represented principle of organic and conventional growing, like as first research factor and application of microbiological fertilizers is the second research factor. Plot is set in four repeated with length lines from 5 m. Each repetition is shared on 10 large plots (5 plots for conventional and 5 plots on organic growing). For sowing were use seed soybean varieties Galina which one is early maturing varieties and belongs to the zero group of ripening and created in Institute of Field and Vegetable growing in Novi Sad. Previous crops for soybean in the previous three years were barley, corn, wheat. Previous crops not fertilized by mineral fertilizers, not used chemical protection for plants in the previous three years. On the surface of plot which is planned for organic production performed the basic fertilization by bovine manure in quantity of 15 tha^{-1}.On part of plot for conventional production for basic fertilization used 100 kg Nha^{-1}. By the manure and nitrogen also used microbiological fertilizer in liquid condition which contains mixture of different varieties microorganisms (bacteria of milk acid, photosynthetic bacteria, yeasts, actinomycetes, fungi). Sowing was performed by machines; six rowing machines in depth of 4 cm. Size of main plot is 15 m^2. Set of plants is 500.000 plants ha^{-1} . Protection crop from weeds was done by cultivating, hand digging and twitch weeds.

Microbiological fertilizer used in both ways of production per lot in five variants; 1-control (with out of used microbiological fertilizers), 2-tretment land seven days before of sowing, 3-in phonological stage 1.-3 leafs, 4-begining of flovering, 5-full of flovering. Reproduction stage, beginning and full of flovering is given in definition Fehr and Caviness (1977). Land treatment was carry out with quantity of 30 lha^{-1} microbiological fertilizer which was diluted with water in quantity of 500 lha^{-1}. It is put in land by rotary hole in depth of 10 cm. In variants with foliar applicant’s preparations was used quantity of 5 lha^{-1} microbiological fertilizer dissolved in 400 l of water.

It is applied with help of back sprayer. Harvest is done by hand, (plants taken from two central row instead of frontal plants and carefully are connected in bundles). Thrashing is done by Wintersteiger combines which is just used for soybean plots in Department of Field and Vegetable growing in Novi Sad. Threshed grain is measured, determined contains of moisture which is reduced to 13% and by this calculated yield per unit area. Contents of protein and oils in grain of soybean is stated by helping DA-700 FLEXI-MODE NIR/VIS spectrophotometer (Balesevic-Tubic et al., 2007) in Department of Field and Vegetable growing in Novi Sad. The air temperature and precipitation values are given from

Agricultural extension service, Backa Topola. Data are determinate by analysis variance according to the method two factor split –plot gate, differences between treatments tested by LSD-test. The correlation coefficients between tested properties of soybean were calculated. For statistical analysis of the results was used to program GENSTAT 9.1 (Trial Version) and Statistical 12.0.

Results and Discussion

Chemical properties of chernozem represent special value of this kind of soil (Tab. 1). $CaCO_3$ is present in this soil from its surface, but in AC and C horizon it is present in great amounts. Because of that, in humus accumulative horizon chernozem has neutral, and at greater depth weak alkaline reaction.

Table 1. Chemical properties of chernozem (Backa Topola, Serbia)

Horizons	Depth	pH		$CaCO_3$	Humus	N	mg/100g soil	
	(cm)	KCl	H_2O	(%)	(%)	(%)	P_2O_5	K_2O
A arable	0-35	7.01	8.05	1.95	2.63	0.173	11.2	20.5
A subarable	35-55	7.20	8.25	3.79	2.03	0.134	7.9	17.2
AC	55-95	7.79	8.63	19.19	1.60	0.105	2.9	10.5
C	95-200	8.05	8.75	32.12	0.36	0.024	2.4	6.6

According to its requirements, soybean can be classified in plants which require higher temperatures and at high temperatures are well-developed, but also, soybean is resistant to short periods of low temperatures and frost. In flovering phase soybean is sensitive to low soil moisture, and o low relative humidity, especially if drought occurs at the same time with high temperatures (Sekulic and Kurjacki, 2008). In both year average month temperatures during vegetation were at the multy-year average (Fig. 1) and they were appropriate to soybean requirements during the grow and development phases. Precipitation significantly deviated from multy-year average for vegetation period from April to September. In 2012 precipitation sum was lower for 24.4 % (82.1 $l.m^{-2}$), and in 2013 it was higher for 43.5 % (146.4 $l.m^{-2}$) compared to multy-year average. However, precipitation distribution was better in 2012 because there were more precipitation in July compared to 2013 and multy-year average. Then soybean was in flovering phase and pod formation, when soybean needs the most the soil moisture. Too much water in stages from germination to flovering, as it was the case in 2013, plants form big aboverground mass and relatively shallow root system, which reduced plant’s resistence to drought. For soybean it is often more important distribution of precipitation than its sum during vegetation (Dozet, 2006, 2009, Djukic, 2009). Average relative humidity significantly deviated from multy-year average and in 2012 it was just 55 %, while in 2013 it was 63 %. Relative humidity wasn’t favorable for soybean in its reproductive phases.

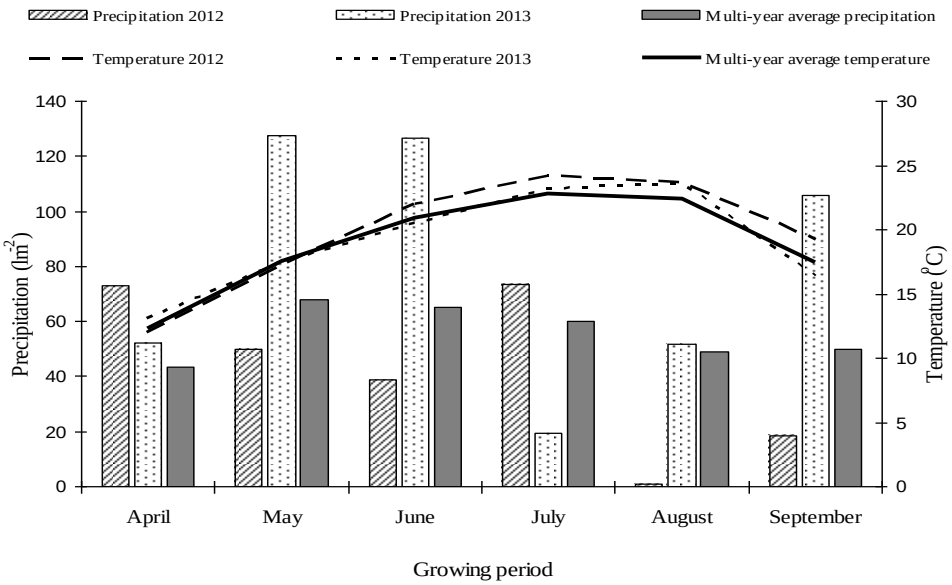

Figure 1. Weather conditions during the production year and multi-year average

Table 2. Proteins and oil in the soybean depending on cultivation technology and application of microbiological fertilizer (%)

Year	Microbial fertilizer (B)	Variants	Protein content Method of production (A) Conventional	Organic	x̄ (B)	Oil content Method of production (A) Conventional	Organic	x̄ (B)
2012		1	40.40	40.24	40.32	20.97	21.09	21.03
		2	40.92	37.04	38.98	20.25	23.77	22.01
		3	40.47	40.35	40.41	21.13	20.57	20.85
		4	39.97	41.37	40.67	21.55	20.33	20.94
		5	39.82	41.42	40.62	21.93	19.55	20.74
		x̄ (A)	**40.32**	**40.08**	**40.20**	**21.17**	**21.05**	**21.11**
2013		1	39.89	40.55	40.22	21.57	21.97	21.77
		2	41.29	41.01	41.15	19.56	20.40	19.98
		3	40.08	39.92	40.00	19.78	22.92	21.35
		4	38.78	38.74	38.76	22.15	21.79	21.97
		5	38.02	38.32	38.17	22.03	23.45	22.74
		x̄ (A)	**39.61**	**39.71**	**39.66**	**21.02**	**22.10**	**21.56**
		x̄ 2012-2013	**39.97**	**39.90**	**39.93**	**21.09**	**21.58**	**21.33**

		Protein content Factors A	B	AxB	BxA	Oil content Factors A	B	AxB	BxA
2012	$LSD_{0.01}$	0.93	0.23	1.63	0.43	0.60	0.17	1.04	0.30
	$LSD_{0.05}$	0.70	0.19	1.23	0.33	0.45	0.13	0.78	0.23
2013	$LSD_{0.01}$	0.98	0.28	1.70	0.49	0.50	0.27	1.14	0.40
	$LSD_{0.05}$	0.74	0.21	1.28	0.37	0.35	0.23	0.88	0.33

An amount of average contents proteins is 39.93%, in conventional production was higher than in organic cropping system (Tab. 2). Influence of treatment by different technology in production of soybean was not showed regularity in measured values. They don’t have common statistical differences. Using microbiological fertilizers shows significant impact in contents of proteins in grains. In 2012 highest contents of proteins was registered on 4.

variants and it was very important in comparing with other variants, instead of 5. Variants. In 2013 on 5. Variants determined the lowest percentage of proteins in soybean grains in compare with control of rest of tested variants and by highest level of statistic relevancy. Measured average oil content which is 21.33%, but in 2012 was lower (21.11%) in compare in 2013.year (21.56%). On average for both years recorded highest percentage in soybean grains which was in organic productions (21.58%). For 2.32% higher than conventional productions (21.09%) (Tab. 2). In first researching year wasn't been significance differences in tested between applied growing technology.

However in 2013 significantly higher contents of oil recorded in organic productions and it is for 5.02% in compare with conventional productions. In research Djukic et al. (2015) using of microbiological fertilizer (effective microorganisms) fortified significant increased microbiological activity, fortified a higher total number of microorganisms and Azobacter in compare with control.

Table 3. Correlative interdependence of some studied properties of soybean

Observed traits	HP	PN	GN	GW	W1000	Z	P	O
HP		0,68*	0,71**	0,60*	-0,29	0,60*	0,45	-0,42
PN	0,68*		0,88**	0,39	-0,48	0,39	0,46	-0,37
GN	0,71**	0,88**		0,33	-0,71**	0,33	0,36	-0,21
GW	0,60*	0,39	0,33		0,38	0,78**	0,87**	-0,85**
W1000	-0,29	-0,48	-0,71**	0,38		0,38	0,31	-0,48
Y	0,60*	0,39	0,33	0,78	0,38		0,87**	-0,85**
P	0,45	0,46	0,36	0,87**	0,31	0,87**		-0,93**
O	-0,42	-0,37	-0,21	-0,85**	-0,48	-0,85**	-0,93**	

$p<0.05$* $p<0.01$**

Number of pods (PN) was in a very positivley correlation in number of grains (GN), and number of grains (GN) with plant height (PH) (Table 3). Yield (Y) was in a strong correlation between mass of grain (GW) and contains protein (P), but it was calculated a strong negative correlation between number of grains (GN) and weight of 1000 grains (W1000), such as and between grain yield (Y), hawever contains protein (P) and oil in grain (O) (-0.85 and -0.93). Similar results got Djukic (2009), Dozet i sar. (2014).

Conclusion

Application of growing technology not significant effect in contains of protein and oil in grain of soybean. These properties are conditioned by the genetically. Using of microbiological fertilizer has been significant impact depending on the time of using. But is not recorded appropriate regularity, and because of it plot required repeat on some or different region. Correlative dependence between important tested properties was been statistically important.

Ecological soybean production, as production system with respect of environmental principles and standards, as well as specific local agroecological conditions, has its priority.

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COMPARISON OF DIFFERENT CROP SEQUENCES AND THEIR INFLUENCES ON MAIZE GROWING PARAMETERS AND YIELD

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Abstract

Crop sequences in crop rotation can be very important factor and can influence on different parameters. In this paper we compare two type of three-crop rotation with different crop sequence. We would like to find out on which way crop sequence influence on LAI, maize height and grain yield.

Experiment was set up 2009 on Maize Research Institute “ZemunPolje”. First crop sequence was wheat-soybean-maize (WSM) and second was soybean-wheat-maize (SWM). Difference between two crop rotations was only previous crop of maize and in first rotation it was soybean and in second it was wheat. We have sown hybrid ZP 606. In both crop rotation we had herbicide treatment: with herbicide application (B1) and without herbicide application (B2), it was weedy check. We measured LAI, maize height and grain yield.

In 2012, LAI and grain yield were higher in SWM then in WSM rotation in all treatments. Only, maize height was higher in WSM in weedy check in comparison with SWM rotation in same treatment, but it was not statistically significant.

Soybean-wheat-maize rotation is much better for achieving higher growing parameters and grain yield of maize. Always should sow wheat as previous crop for maize, because wheat as narrow crop, decreasing negative influence of weeds, insects and other pests.

Key words: *maize, crop rotation, LAI, grain yield.*

Introduction

The application of the crop sequence, and therefore the crop rotation, has positive effects on physical, chemical and biological properties of soil (Aziz et al., 2011). Moreover, soil is less compacted (Seremesic et al., 2008), and weed infestation can be reduced (Dolijanovic et al., 2011). Today, the crop sequence is one of important cropping practices, due to which optimum maize yields can be achieved without adverse effects on environments (Pop et al., 2009).

Various effects on crops can be achieved by the application of the certain growing system. Since more intensive cultivation of legumes, first of all of soybean, was initiated, a new type of crop sequence (maize-soya bean-wheat) has been applied. The presence of plants belonging to the family *Fabaceae*significantly contributes to the efficiency of the crop rotation, because the maize grain yield has been increasing, while the application of nitrogen fertilisers has been decreasing (up to 50%), which is a significant saving, and is important for soil conservation (Videnovic et al., 2007). Therefore, long-term crop rotations that include legumes are much more agronomical acceptable than the short-term crop rotations (Stranger and Lauer, 2008).

Three crop rotation, where are rotating maize, wheat and soybean have two type of crop sequences. In one soybean is previous crop for maize and in second wheat is previous crop. The role of previous crop is significant for maize (Idikutand Kara, 2011).These crops leave different amount of nutrients and weed seeds in soil, so that can influence on productivity of maize. If we have enough amounts of nutrients in soil and if they are well distribute and balance it will help to achieve higher leaf area and grain yield (Milosev et al., 2002). Higher

value of leaf area index (LAI) in early stage of development directly influence on grain yield increasing (Thornley, 2000).Increasing and improving of maize grain yield depend on increasing of harvest index and crop biomass (Sinclair, 1998). But, relation between leaf area and grain yield can vary depend of biotic and abiotic factors and can make problem to understand their interaction (Milosev et al., 2008).
Height of plants can be very important factor for prediction of grain yield. Relation between maize height and grain yield are in very strong positive correlation in stage when upper maize leaves are completely developed (Liu and Wiatrac, 2011).Cob height and plant height are in positive correlation with grain yield of maize (Golam et al., 2011).
So the aim of this paper was to investigate influence of different crop sequences on LAI index, height and grain yield of maize.

Material and methods

The experiment was set up at experimental field of Maize Research Institute ZemunPolje Belgrade (44° 52' 00" N 20°19' 00" E)in 2009. We investigated influence of three-crop rotation with different crop sequences. First crop sequence was soybean-wheat-maize rotation (SWM) and second was wheat-soybean-maize rotation (WSM). In first year (2009) and fourth year (2012) of experiment on both plots we sown maize hybrid ZP 606. In both crop rotation, we had herbicide treatment: with herbicide application (B1) and without herbicide application (B2), it was weedy check. We measured LAI, maize height and grain yield. In this paper we show results of maize from first year (2009) and after first rotation (2012) of maize, soybean and wheat in two different crop sequences.
Weather conditions were completely different in these two years (Table 1). In 2009, average monthly temperatures were lower in Jun, July and August with much more precipitation than in 2012, so on all measured parameters were lower in 2012 than in 2009. This 2012 year was very unfavorable for maize production.

Table 1.Average monthly temperatures and sums of precipitation in 2009 and 2012 in ZemunPolje.

Months	**Monthly temperatures**		**Sums of precipitations**	
	2009	**2012**	**2009**	**2012**
March	8.6	10.1	63.5	2.8
April	16.2	14.4	5.6	66.7
May	19.8	17.9	35.0	127.5
Jun	21.2	24.6	153.0	13.9
July	24.0	27.1	79.6	39.4
August	24.1	26.2	44.8	4.0
September	21.1	22.1	4.6	31.4
October	13.8	15.4	101.8	52.5
Average/Sum	**18.6**	**19.7**	**487.9**	**338.2**

Results and Discussion

If plant achieve LAI of 3.5-4.0 m^2/m^2 till tasseling stage, potentially yield mostly increasing (Jones, 2002). Higher leaf area of maize, make possible better using of sun light and so increase grain yield of maize (Ammanullah et al., 2009).
The plant height depends on the sowing density and rates of applied nitrogen fertilisers. According to results obtained by Hassan et al. (2000) the greater sowing density was the greater plant height, internode length and the ear height were. Between the plant height and

the grain yield of maize exist positive relationship (Spasojevic et al., 2013). Crop rotation has positive effect on increasing of LAI, height and grain yield of maize (Spasojevic, 2014).
Based on results from first year of experiment (2009), LAI, height and grain yield had, more or less, same values in WSM and SWM crop sequences (Table 2). But after first rotation of maize, soybean and wheat (2012), mostly we got higher values in SWM in B1 and B2 treatment with herbicide then in WSM rotation (Table 2). In SWM rotation, LAI was higher 0.92 m^2/m^2 (B1) and 0,33m^2/m^2 (B2) in comparison with WSM rotation. In B1, maize plants were higher in SWM, in average, for 21.7 cm, but in B2 plants were higher in WSM rotation for 3.3 cm. And finally, higher grain yield we achieved in SWM rotation, in average, for 1.09 t/ha (B1) and for 0.04 t/ha (B2) than in WSM, respectively.

Table 2. Influence of crop sequences on LAI, height and grain yield of maize

Quantity of herbicides	2009		2012	
	SWM	WSM	SWM	WSM
LAI (m^2/m^2)				
B1	3.76	3.78	3.90	2.98
B2	2.62	2.97	2.24	1.91
Height (cm)				
B1	253.5	246.5	197.5	175.8
B2	214.3	235.0	142.3	145.6
Grain yield (t/ha)				
B1	14.34	14.00	6.97	5.88
B2	12.32	13.98	4.15	4.11

	LAI	Height	Grain yield
$LSD_{0,05}$	0.42	7.1	0.56

All these differences between SWM and WSM, were statistically significant in B1 treatment, but in B2 we did not have statistically significant differences. That we have had better weather condition, maybe we have got bigger differences, especially in B2 treatment, and they have been statistically significant.
One of the most important reasons, why we got these results, can be previous crop, because all the other cropping practices are same. In SWM rotation wheat was previous crop for maize and in WSM rotation soybean was previous crop. The same results were got Bly et al. (2004) who were investigated influence of tillage method and previous crop on height and grain yield of maize. Mean corn plant heights following wheat were significantly taller when compared to soybeans as the previous crop and corn grain yield means were less following soybeans compared to wheat. These could be due to storage of more soil moisture after wheat compared to soybean, especially since the wheat was not harvested. Sims (2007) concluded that wheat is much better previous crop for sugar beet and he found out that when sugar beet is grown after a crop of soybean, root yields were still greater than when following corn but, less than following wheat. The findings of this research indicate that growing sugar beet after a wheat crop is consistently the better rotation of the three previous crops tested (wheat, soybean and maize). Wheat and other small grains species, achieved increasing in soil organic matter resulting in improved soil structure and increased water infiltration, disruption of pest cycles, and the addition of growth-promoting substances (Dogan and Bilgili, 2010).
Beside it, wheat can decrease weed infestation for following crop. Wheat as preceding crop, alongside with herbicides applied during the growing season as well as glyphosate-based herbicides applied to stubble fields after wheat harvest, greatly contribute to the reduction of weed infestation of maize as the following crop (Spasojevic et al., 2014). Wheat seedlings and

straw have allelopathic effects on the increase of weed plants (Wu et al., 1998). Small grains due to a higher growing density can suppress many weeds, particularly some perennial species, resistant to other control measures (Stefanović et al., 2011).

Conclusion

With application of soybean-wheat-maize crop sequence is possible to achieve higher maize plants, leaf area and grain yield than with application of wheat-soybean-maize crop sequence. Wheat is much better previous crop in comparison with soybean for maize production, because forming better soil conditions, leaving less weed seeds and reproductive organs and higher quantity of growth-promoting substances. We must take into consideration that in this experiment finished only one rotation and for precise results is necessary done few rotations, so we will continue with this experiment.

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PHYSICAL ATTRIBUTES OF PLUM FRUIT AT PHYSIOLOGICAL AND HARVEST MATURITY

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Abstract

During 2011 and 2012, differences in the physical properties of fruit at physiological maturity vs. harvest maturity were evaluated in nine plum cultivars (‘Boranka’, ‘Čačanska Rana’, ‘Čačanska Lepotica’, ‘Valerija’, ‘Timočanka’, ‘Čačanska Najbolja’, ‘Čačanska Rodna’, ‘Stanley’ and ‘Mildora’) grown in high density planting system under the environmental conditions of Čačak. In addition to recording the dates of the two maturity stages, the study involved measurement of fruit weight, three fruit linear dimensions (length, width, thickness) for fruit volume and shape index calculation, and soluble solids content (SSC). Results showed that harvest maturity in the tested cultivars occurred 6.28 days after physiological maturity. The number of days from physiological maturity to harvest maturity varied between 2.5 in ‘Čačanska Rana’ to 9 in ‘Čačanska Lepotica’ and 9.5 in ‘Čačanska Najbolja’. During this period, fruit length increased by 6.39%, width by 7.03% and thickness by 5.58% on average. When analysed across cultivars, the highest increase in fruit length, width and thickness was observed in ’Čačanska Najbolja’ (12.72%; 17.16% and 14.98%, respectively). Fruit shape index did not significantly change between the maturity stages. The average increase in fruit weight and volume was 18.24% and 16.81%, respectively, with the measured values ranging from 5.65% and 6.39% (‘Valerija’) to 41.08% and 29.69% (‘Čačanska Najbolja’). SSC in plum fruit was 12.26% higher at harvest maturity than at physiological maturity. The lowest increase in SSC was found in ‘Čačanska Rana’ (6.26%) and the highest in ‘Timočanka’ (22.89%).

Key words: *plum, physiological maturity, harvest maturity, fruit properties*.

Introduction

The plum (*Prunus domestica* L.) is the most common fruit crop in Serbia. Its fruits are used fresh, processed and dried (Milošević and Milošević, 2012). The fruit has a high water content, and also holds carbohydrates, fibre, pectin, organic acids, minerals, tannins, colouring matter, enzymes and vitamins. The predominant sugards include sucrose, glucose and fructose (Forni et al., 1992), and malic, tartaric and citric acids are the predominant organic acids. Physical and chemical attributes of plum fruits differ across cultivars, environmental conditions and growing methods (Nergiz and Yildiz, 1997; Usenik et al., 2008). Moreover, differences are also largely dependent on the degree of fruit maturity and harvest date (Nunes et al., 2009; Singh et al., 2009). Internal and external fruit quality parameters change during fruit ripening (Unuk et al., 2011). Understanding changes in the physical and chemical properties of the plum fruit during ripening i.e. from physiological maturity to harvest maturity, is of high importance in determining the optimum harvest time. Harvest time also causes substantial postharvest changes in fruit properties and affects fruit storage life (Taylor et al., 1995). Consumer acceptance of a plum cultivar is mostly related to its fruit weight and fruit physical and organoleptic attributes (Crisosto et al., 2007).

The objective of this study involving nine plum cultivars was to determine changes in major physical properties of the fruit at both physiological and harvest maturity.

Material and methods

The orchard trial with nine plum cultivars was established at the village of Gornja Gorevnica near Cacak (43°53'N latitude; 20°21'E longitude; 390 m a.s.l.), Western Serbia. Trees were planted in High Density Planting (HDP) at a spacing of 4 m × 2 m (1.250 trees ha^{-1}) and trained to the Spindle Bush system. Orchard management was consistent with standard practice for HDP, except irrigation. Summer pruning was used. In addition, climatic conditions in both years were similar.

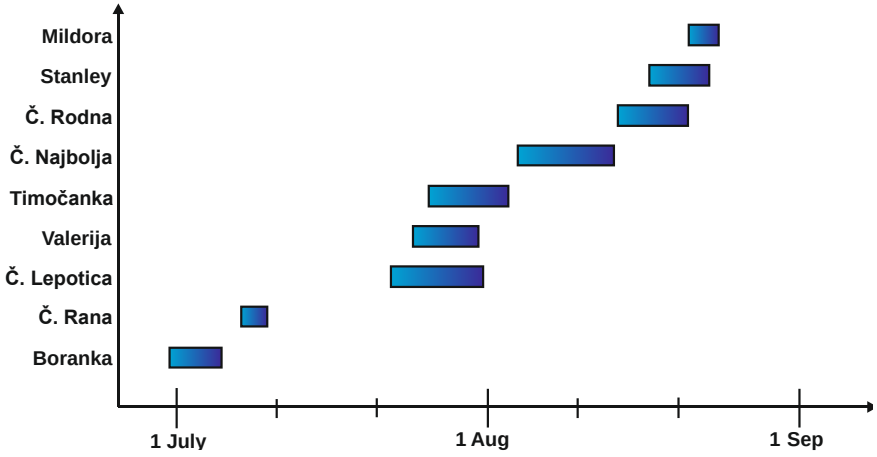
During 2011 and 2012, the research involved the following plum cultivars: 'Boranka', 'Čačanska Rana', 'Čačanska Lepotica', 'Valerija', 'Timočanka', 'Čačanska Najbolja', 'Čačanska Rodna', 'Stanley' and 'Mildora'. The Čačak area is well-known for plum cultivation. The tested cultivars (except 'Stanley') were created at the Fruit Research Institute, Čačak, and are the most common plum cultivars not only in the Čačak area but also in Serbia and other countries in the region.

In both years, dates of fruit physiological maturity and harvest maturity were recorded. Physiological maturity and harvest maturity were identified using the method specified by Crisosto 1994) and UPOV (2002). Fruit linear dimensions (length, width and thickness) were measured using an electronic digital caliper (WOMAX – Germany). Based on fruit length, width and thickness, the fruit shape index was calculated using the expression: Fruit Shape Index = $Length^2$ (Width × Thickness)$^{-1}$. Fruit volume was measured as follows: A graduated cylinder (PolyLab, 1000 ml) was filled with water to a certain level. The fruits were placed into the water one by one and their volume read as the equivalent of the difference in the water level in the cylinder before and after the fruit was submerged. Fruit weight was measured on a KERN FCB technical balance (Kern & Sohn GmbH, Belingen, Germany), weighing range 6100 g, accuracy ±0.2 g. The fruit juice produced by crushing and squeezing the fruit was analysed for soluble solids (^{o}Brix) using an MT 032 ATC manual refractometer (Turoni & C., Forlì, Italy). All measurements were made in 3 replicates with 10 fruits per replicate.

Statistical analysis and testing for the significance of differences were performed by the analysis of variance and the LSD test at $P \leq 0.05$, respectively, using the ANOVA statistical software (SPS Statistica, Software 5.0).

Results and discussion

The results on the difference between physiological and harvest maturity dates for plum cultivars are presented in Graph 1. The graph shows average values for 2011 and 2012.



Graph. 1. Physiological and harvest maturity dates for plum fruits

On average, harvest maturity of the fruit occurred 6.28 days after the physiological maturity date. Shorter than average intervals were observed in 'Boranka', 'Čačanska Rana',

‘Valerija’, ‘Stanley’ and ‘Mildora’, and longer periods in ‘Čačanska Lepotica’, ‘Timočanka’, ‘Čačanska Najbolja’ and ‘Čačanska Rodna’. The shortest period between physiological maturity and harvest maturity was registered in ‘Čačanska Rana’ and ‘Mildora’ (2.5 and 3 days). In contrast, the highest number of days from physiological maturity to harvest maturity was in ‘Čačanska Lepotica’ and ‘Čačanska Najbolja’ (9 and 9.5 days). Although fruit skin colour is not a realiable indicator of maturity (Walkowiak-Tomczak et al., 2008; Unuk et al., 2011), it is among the major indicators of physiological maturity. As confirmed by the present study, the fruits of ‘Čačanska Lepotica’ and ‘Čačanska Najbolja’ typically turn blue very early and enter the stage of physiological maturity relatively early (Milošević, 2002).

The length, width and thickness of plum fruit at both physiological and harvest maturity in the tested plum cultivars are given in Table 1.

Tab. 1. Length, width and thickness of plum fruit at physiological and harvest maturity

	Length (mm)			Width (mm)			Thickness (mm)		
	PM	HM	D (%)	PM	HM	D (%)	PM	HM	D (%)
Cultivar (A)									
Boranka	38.9±1.6	40.0±1.9	2.83±0.1 d	36.3±1.5	37.5±1.7	3.30±0.2 e	33.9±1.1	34.2±1.7	0.88±0.1 e
Čačanska Rana	50.3±2.7	52.0±2.0	3.37±0.1 d	38.5±1.9	40.8±1.9	5.97±0.4 d	36.3±1.8	38.1±1.9	4.95±0.2 d
Čačanska lepotica	39.8±1.5	43.3±3.0	8.79±0.3 b	34.6±1.1	38.3±1.7	10.69±0.9 c	32.9±1.7	35.2±2.0	6.99±0.3 c
Valerija	43.8±2.1	45.7±2.1	4.33±0.2 cd	41.9±2.2	43.6±2.0	4.05±0.3 de	43.2±1.9	44.0±1.9	1.85±0.1 e
Timočanka	46.3±2.0	50.6±2.1	9.28±0.4 b	40.0±1.9	45.9±2.5	14.75±1.1 b	39.5±1.9	41.8±2.0	5.82±0.2 cd
Čačanska Najbolja	44.8±2.1	50.5±1.9	12.72±0.5 a	36.7±1.3	43.0±2.1	17.16±1.1 a	32.7±1.6	37.6±1.9	14.98±1.1 a
Čačanska Rodna	39.7±1.1	41.5±1.8	4.53±0.2 c	30.4±1.0	30.9±1.8	1.65±0.1 f	28.4±0.9	28.8±1.5	1.40±0.1 e
Stanley	43.6±1.9	45.7±2.2	4.81±0.3 c	31.5±1.7	33.1±1.7	5.07±0.2 d	29.7±1.1	32.6±1.8	9.76±0.4 b
Mildora	36.3±1.1	38.8±1.4	6.88±0.3 c	30.5±1.6	30.7±1.1	0.65±0.1 f	27.7±1.0	28.7±1.6	3.61±0.1 d
Year (B)									
2011	43.3±2.0	46.0±2.1	6.23±0.3	36.0±1.2	38.4±1.9	6.67±0.5	34.4±1.5	36.1±2.0	5.14±0.3 b
2012	41.9±1.6	44.7±1.9	6.55±0.3	35.2±2.0	38.0±1.7	7.39±0.5	33.2±1.3	35.1±1.6	6.02±0.3 a
ANOVA									
A			**			**			**
B			ns			ns			*
A×B			ns			ns			ns

The different lowercase letter(s) in columns indicate significant differences among means within each cultivar at $P \leq 0.05$ by LSD test. PM = physiological maturity; HM = harvest maturity; D = difference between harvest maturity and physiological maturity (%)

The analysis of variance showed a very significant effect of cultivar on differences in fruit length, width and thickness between physiological and harvest maturity stages. Fruit thickness was also significantly affected by year as a variability factor.

The greatest change in fruit length and width between physiological and harvest maturity stages was observed in ‘Čačanska Najbolja’, ‘Timočanka’ and ‘Čačanska Lepotica’, whereas ‘Čačanska Najbolja’, ‘Stanley’ and ‘Čačanska Lepotica’ exhibited the greatest change in fruit thickness. The highest increase in fruit length, width and thickness was found in ‘Čačanska Najbolja’ (12.72±0.5%, 17.16±1.1% and 14.98±1.1%, respectively). The smallest change in fruit length and thickness between physiological and harvest maturity stages was exhibited by ‘Boranka’ (2.83±0.1%, 0.88±0.1%, respectively), and the smallest change in fruit width by ‘Mildora’ (0.65±0.1%). Apart from these cultivars, ‘Čačanska Rodna’ also showed small changes in fruit linear dimensions between physiological and harvest maturity stages. The findings also suggest that the cultivars that produce small sized fruit (‘Čačanska Rodna’ and ‘Mildora’) and those that went through a short period between physiological maturity and harvest maturity (‘Mildora’ and ‘Boranka’) exhibited a smaller increase in fruit length, width and thickness during the period. In contrast, the cultivars that produce large sized fruit ‘Čačanska Najbolja’, ‘Timočanka’ and ‘Čačanska Lepotica’ showed the highest increase in fruit length, width and thickness. Although producing large sized fruit, ‘Čačanska Rana’ exhibited no significant increase in fruit dimensions due to the very short period between physiological and harvest maturity stages.

Plum cultivars generally attain maximum fruit dimensions at full i.e. harvest maturity, when they also exhibit their cultivar-specific characteristics (Ionica et al., 2013). This finding was confirmed by the present study.

Changes in the fruit shape index, fruit volume and fruit weight in plum cultivars between physiological and technological maturity stages are presented in Tab. 2.

Tab. 2. Fruit shape index, fruit volume and fruit weight at physiological and harvest maturity

Treatment	Fruit shape index			Fruit volume (cm^3)			Fruit weight (g)		
	PM	HM	D (%)	PM	HM	D (%)	PM	HM	D (%)
Cultivar (A)									
Boranka	1.23±0.1	1.24±0.1	+0.81±0.1 c	30.9±1.5	33.1±1.7	6.81±0.2 d	30.5±1.7	32.6±2.2	6.91±0.3 e
Čačanska Rana	1.81±0.1	1.74±0.1	-3.86±0.2 b	43.4±2.8	51.3±2.2	18.23±0.9 b	44.4±2.9	50.2±3.2	13.08±0.9 d
Čačanska lepotica	1.39±0.1	1.39±0.1	0.00±0.0 c	30.1±1.9	38.3±1.9	26.81±1.3 a	28.2±1.7	37.8±2.1	34.18±2.7 b
Valerija	1.06±0.1	1.08±0.1	+1.88±0.1 c	49.1±3.1	52.3±2.9	6.39±0.7 d	49.5±3.7	52.3±4.1	5.65±0.4 e
Timočanka	1.35±0.1	1.33±0.1	-1.48±0.1 c	47.1±4.0	61.2±2.6	26.51±1.1 a	47.2±4.0	58.7±4.0	24.50±1.1 c
Čačanska Najbolja	1.67±0.1	1.58±0.1	-5.38±0.2 ab	34.5±2.1	44.7±2.0	29.69±2.0 a	33.9±1.8	47.9±3.7	41.08±2.7 a
Čačanska Rodna	1.82±0.1	1.96±0.1	+4.39±0.2 b	21.1±0.9	22.6±1.1	6.89±0.2 d	24.5±1.1	27.2±1.3	11.16±0.8 d
Stanley	2.03±0.1	1.93±0.1	-4.92±0.3 b	28.7±1.4	31.9±2.0	11.00±0.9 c	29.8±2.0	34.4±1.9	15.40±1.1 d
Mildora	1.56±0.1	1.70±0.1	+8.97±0.5 a	18.4±0.8	21.9±1.6	19.03±1.1 b	21.6±1.2	24.3±1.7	12.26±1.0 d
Year (B)									
2011	1.58±0.1	1.55±0.1	-1.89±0.3	35.7±2.7	40.4±1.6	13.16±0.8	35.8±1.9	41.8±2.6	16.76±1.1
2012	1.56±0.1	1.55±0.1	-0.64±0.1	31.7±2.1	38.8±2.7	22.39±1.6	33.0±0.6	39.4±2.4	19.39±1.9
ANOVA									
A			**			**			**
B			ns			ns			ns
A×B			**			ns			ns

The different lowercase letter(s) in columns indicate significant differences among means within each cultivar at $P \leq 0.05$ by LSD test. PM = physiological maturity; HM = harvest maturity; D = difference between harvest maturity and physiological maturity (%)

In terms of the fruit shape index, very significant interactions were observed between cultivar and year. Overall, no significant changes in the fruit shape index were found between physiological and harvest maturity stages.

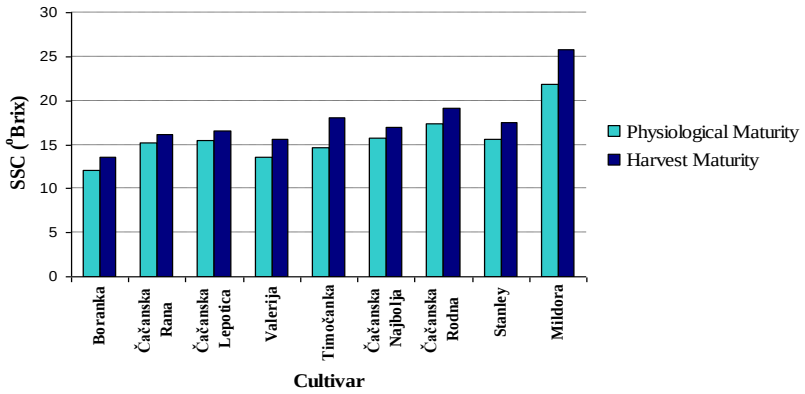
As for the change in fruit volume and weight, the analysis of variance revealed a highly significant effect of cultivar.

Fruit volume between physiological and harvest maturity stages increased in all plum cultivars. The highest increase was in ‘Čačanska Najbolja’ (29.69±2.0%), followed by ‘Čačanska Lepotica’ (26.81±1.3%) and ‘Timočanka’ (26.51±1.1%), and the lowest in ‘Valerija’ (6.39±0.7%), ‘Boranka’ (6.81±0.2%) and ‘Čačanska Rodna’ (6.89±0.2%).

A similar pattern was observed in fruit weight. The highest increase in fruit weight between physiological and harvest maturity stages was, as with fruit volume, in ‘Čačanska Najbolja’ (41.08±2.7%), followed by ‘Čačanska Lepotica’ (34.18±2.7%) and ‘Timočanka’ (24.50±1.1%), and the lowest in ‘Valerija’ and ‘Boranka’ (5.65±0.4% and 6.91±0.3%, respectively).

Changes in fruit weight occur even after harvest depending on storage conditions and length of storage (Khan et al., 2013). Nevertheless, fruit weight undergoes the most substantial change during the last several days to its full i.e. harvest maturity (Milošević, 2002), which was confirmed by the present results.

Changes in soluble solids content of plum fruit at physiological and harvest maturity are presented in Graph 2.



Graph. 2. Soluble solids content of plum fruit at physiological and harvest maturity

In all tested cultivars, the average increase in the fruit soluble solids content between physiological and harvest maturity was 12.26±1.1%. The highest increase was in ‘Timočanka’ and ‘Mildora’ (22.89±1.4% and 18.21±0.3%, respectively), and the smallest change in ‘Čačanska Rana’ and ‘Čačanska Lepotica’ (6.26±0.4% and 6.99±0.5%, respectively).
Fruit maturity can be defined as the stage of development giving minimum acceptable quality for the ultimate consumer (Crisosto, 1994). Soluble solids content is a very important parameter in identifying the optimal harvest ripeness of fruit (Usenik et al., 2014), and it shows signficant differences both across cultivars and in the same cultivar during fruit ripening. Certain cultivars can attain some cultivar-specific traits (colour) much before they reach an adequate soluble solids content (Unuk et al., 2011). This result was confirmed by the present findings.

Conclusion

The results presented in this study suggest the following:
The number of days from physiological maturity to harvest maturity varied between 2.5 in ‘Čačanska Rana’ to 9.5 in ‘Čačanska Najbolja’.
During the period, the highest increase in fruit linear dimensions (length, width and thickness) was observed in ‘Čačanska Najbolja’, and the lowest in ‘Boranka’ and ‘Mildora’. The fruit shape index did not significantly change at harvest maturity compared to physiological maturity. Fruit volume and fruit weight exhibited the highest increase at harvest maturity in relation to physiological maturity in ‘Čačanska Najbolja’, ‘Timočanka’ and ‘Čačanska Lepotica’, whereas the lowest increase was measured in ‘Valerija’ and ‘Boranka’. As for the change in the soluble solids content, the highest increase was found in ‘Timočanka’ and ‘Mildora’, and the lowest in ‘Čačanska Rana’ and ‘Čačanska Lepotica’.

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BIOLOGICAL AND POMOLOGICAL PROPERTIES OF PROMISING PLUM HYBRIDS CREATED AT THE FRUIT RESEARCH INSTITUTE–ČAČAK, SERBIA

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Abstract

This paper presents the results of a three-year study of ripening time and major physical and chemical properties of fruits of six promising plum (*Prunus domestica* L.) hybrids created at the Fruit Research Institute, Čačak. One of these hybrids was released as a new cultivar and was named ‘Nada’. The cultivar ‘Čačanska Lepotica’ served as the standard. The genotypes under study ripened between 29 July (hybrid 38/62/70) and 2 September (hybrid 22/17/87). An elongated fruit shape, a yellowish-green flesh colour and a firm fruit prevailed, whereas violet blue and dark blue skin colours were equally present. Fruit weight ranged from 26.96±0.68 g (22/17/87) to 56.92±2.87 g (38/62/70), with higher values obtained in 38/62/70 and the new cv.‘Nada’, compared to the standard. The highest flesh/stone ratio was found in hybrid 38/62/70 (96.91±0.19%), which was the only significantly higher result compared to the control cultivar. The highest soluble solids content was measured in hybrid 22/17/87 (17.01±0.96%), and the lowest in hybrid 38/62/70 (11.39±0.44%). Compared to the standard, higher values of soluble solids were exhibited by ‘Nada’ and hybrids 32/21/87, 34/41/87 and 22/17/87. Total acid levels were highest in hybrid 32/21/87 (1.42±0.05%), and lowest in ‘Nada’ (0.35±0.02%). Compared to the standard, higher levels were found in fruits of hybrids 38/62/70, IV/63/81 and 32/21/87. The ratio of soluble solids to total acids was highest in ‘Nada’ (43.72±1.47) and lowest in hybrid 32/21/87 (10.25±0.58). Compared to the control cultivar, higher values of this parameter were observed in ‘Nada’, and hybrids 34/41/87 and 22/17/87. The results of this research suggest that ‘Nada’ can be singled out among the tested genotypes as having the best combination of positive traits.

Key words: plum, hybrid, cultivar, fruit properties

Introduction

Breeding of continental fruit species has been developed in continuity at the Fruit Research Institute in Čačak, since its establishment in 1946, to date (Ogašanović *et al*, 1996). The most prominent achievements and the best results belong to the domestic plum (*Prunus domestica* L.). During the period spanning over six decades, 15 plum cultivars have been created (Paunović *et al.,* 2011). The initial breeding objectives were governed by the imperative to create table cultivars and cultivars with combined properties (Ogašanović *et al.,* 2006). Further expansion of breeding objectives went in the direction of developing resistance to the Plum Pox virus (Ranković *et al*., 1994), enhancing the level of adaptability to the conditions of temperate climate, regular and high cropping, harmonic taste, good transportability of the fruits and their multi-purpose utilisation value (Milenković *et al*., 2006). The initial selection of promising hybrids from the population of hybrid seedlings was performed based on analysis of their most significant biological and pomological properties after the start bearing, for three consecutive years. Further selections were performed among promising hybrids, based on results of many-year comparative studies involving best standard cultivars, aiming to select the genotypes with the best combinations of positive properties.

The aim of this study pertains to analysis of the most important biological and pomological properties of six promising plum genotypes that were created within different breeding programmes, through comparative studies involving cultivar ‘Čačanska Lepotica’.

Material and methods

The experimental research was conducted at the plantation of plum of the Fruit Research Institute in Čačak, located at approximately 4 km to the north of Čačak, at the altitude of around 250 m above the sea level. The plantation was set up in spring 2002, using standard one-year-old seedlings grafted on a Myrobalan rootstock and planted at the 6 x 5 m distance (333 trees per ha^{-1}). The planting was performed using the random block system, with each genotype participating with 15 trees in three replications. The open vase crown was chosen as the training system, and the plantation was treated using standard cultivation measures. The research was conducted during three years (2009–2011). The testing material comprised six promising plum genotypes created at the Fruit Research Institute in Čačak: hybrid 38/62/70 (‘Hall’ × ‘California Blue’); hybrid IV/63/81 (‘Large Sugar Prune’ × ‘Scoldus’); hybrid 32/21/87 (‘Stanley’ × ‘Scoldus’); hybrid 29/29/87 (‘Stanley’ × ‘Scoldus’); hybrid 34/41/87 (‘Valjevka’ × ‘Čačanska Lepotica’) and hybrid 22/17/87 (‘Čačanska najbolja’ × ‘Zelta Boutilcovidna’). Hybrid 29/29/87 was approved as a cultivar in March 2012 and was named ‘Nada’ (Ogašanović *et al*., 2012).

The respective dates of full technological ripeness were monitored and recorded, in order to establish the fruit ripening time. The fruit weight (g) and stone weight (g) were determined by measuring 25 randomly selected fruits and their stones in three replications, using the technical scales (Adventurer Pro AV812M, Ohaus Corporation, Switzerland). The flesh percentage (%) was determined by calculation. Soluble solids content (%) was established using the manual refractometer (Carl Zeiss, Jena, Germany) at room temperature (20°C). Total acids content (%) manifested in malic acid was determined by neutralising the fruit juice using 0.1 N NaOH, with the addition of phenolphtaleine as the indicator of pH value up to 8.1. Based on the obtained data, the ratio between the soluble solids content and total acids was calculated. The fruit shape, skin colour, as well as the colour of the flesh and its firmness was described in accordance with the methodology recommended by UPOV-a (2002). The standard cultivar was ‘Čačanska Lepotica’.

Data analysis was performed using the SPSS statistical software package, Version 8.0 for Windows (SPSS. Inc., Chicago, IL). The variance analysis (ANOVA) was used for establishing the impact of genotype on the fruit weight and stone weight, the flesh percentage, soluble solids content and content of total acids, as well as the ratio between the total soluble solids content and total acids. In the cases when the *F* test was significant, testing of arithmetic means was performed using the test of Least Significant Differences (LSD test) for significance threshold of $P \leq 0.05$.

Results and discussion

The analysed plum genotypes ripened in the period from 29th July (hybrid 38/62/70) and 2nd September (hybrid 22/17/87). An earlier ripening time when compared to cv. Čačanska lepotica was established in hybrid 38/62/70 (Table 1). The average values of fruit and stone weight and the flesh percentage differed significantly depending on the examined plum genotype (Table 1). The average fruit mass of the analysed plum genotypes varied within the range from 25.10±0.89 g (hybrid 34/41/87) to 56.92±2.87 g (hybrid 38/62/70). A larger fruit compared to the standard was determined in hybrid 38/62/70 and the ‘Nada’ cultivar. The largest average stone weight was determined in hybrid 38/62/70 (1.76±0.03 g), which was in accordance with the largest fruit weight, whereas the smallest stone weight was recorded in the standard ‘Čačanska Lepotica’ cultivar (1.34±0.04 g). The only insignificant differences in relation to the standard were established in hybrid IV/63/81. The highest value of flesh percentage was

determined in hybrid 38/62/70 (96.91±0.19%), which was the only significantly higher result in comparison to the control cultivar. The fruit flesh ratio in the ‘Nada’ cultivar was at the level of the standard cultivar, whereas it was significantly lower in all the other genotypes.

Table 1. Harvest time, fruit and stone weight and flesh percentage of plum hybrids.

Genotyp	Harvest time	Fruit weight (g)	Stone weight (g)	Flesh percentage (%)
Hybrid 38/62/70	29 Jul	56.92±2.87 a	1.76±0.03 a	96.91±0.19 a
Hybrid IV/63/81	08 Aug	31.98±0.65 e	1.36±0.05 e	95.75±0.07 c
Hybrid 32/21/87	15 Aug	35.11±1.71 d	1.62±0.01 c	95.38±0.23 d
Nada	19 Aug	45.54±0.29 b	1.68±0.03 b	96.57±0.04 b
Hybrid 34/41/87	29. Aug	25.10±0.89 g	1.43±0.03 d	94.30±0.42 e
Hybrid 22/17/87	02 Sep	26.96±0.68 f	1.46±0.02 d	94.58±0.18 e
Čačanska lepotica	31 Jul	42.24±0.69 c	1.34±0.04 e	96.75±0.06 b

The different lower-case letters assigned to columns show significant differences for $P \leq 0.05$ after applying *LSD* test.

Ripening time and the duration of the fruit development are a specific property of each genotype (García-Mariño *et al*., 2008). Considering the fact that genotypes with either earlier or later fruit-ripening time are generally given preference (Neumüller, 2010; Пауновић *et al*., 2011) it is probably worth noting the potential of the early-ripening hybrid 38/62/70, as well as the lat-ripening ‘Nada’ cultivar and hybrids 34/41/87 and 22/17/87. According to the classification of plum cultivars in relation to the fruit weight as stated by Mišić (1996), hybrids IV/63/81, 32/21/87, 34/41/87 and 22/17/87 represent genotypes with medium-large fruits, whereas hybrid 38/62/70 and cv. ‘Nada’ belong to genotypes with large fruits. The large fruit of hybrid 38/62/70 is a confirmation that cv. ‘California Blue’ appeared as a donor of this property (Mišić, 2002). Hybrid 34/41/87 was characterised by the smallest fruit mass, which contradicted the results previously published by Jakubowski and Lewandowska (2004), where it was reported that cultivar ‘Čačanska Lepotica’ produces progeny with large fruits. The positive impact made by ‘Stanley’ as the mother plant on the fruits weight in the hybrid progeny, as reported by Mišić (2002) and Jakubowski & Lewandowska (2004) is observed in ‘Nada’ cultivar, but not in hybrid 32/21/87. Contrary to results of Blazek and Vávra (2007), and in agreement with the findings of Milošević and Milošević (2011), the positive impact of cultivar ‘Čačanska Najbolja’ on the fruit weight in the hybrid progeny was not confirmed in our studies. The results of our research related to the stone weight have confirmed the previous reports that the mass of stone is considered as a stable cultivar-specific property (Огашановић *et al*., 1996; Depypere *et al*., 2007). The previous research in this field have established a high degree of positive correlation between the stone mass and the mass of fruit (Okut and Akca, 1995), which was partly confirmed by our research. The flesh percentage is considered as an important property of plum (Miletić and Petrović, 1996; Nenadović-Mratinić *et al*., 2007). The same authors have reported the cultivar variability of this property, which is in accordance with the results obtained in our research.

The results of the study related to the fruit shape, skin colour, colour of the flesh and fruit firmness are shown in Table 2. Oval fruit shape was typical for hybrids 38/62/70 and 32/21/87, whereas all the other studied genotypes had an elongated fruit shape, including ‘Čačanska Lepotica’ as the standard cultivar. In addition to the standard cultivar, dark blue colour of the fruit skin was established in hybrids 32/21/87, 34/41/87 and the ‘Nada’ cultivar, whereas hybrids 32/21/87, IV/63/81 and 22/17/87 had a violet- blue colour of the skin. The yellow colour of the flesh was found in hybrid 32/21/87 and the ‘Nada’ cultivar, whereas the remaining genotypes under consideration had a yellowish-green colour of the flesh, which was also present in the standard cultivar as well. Hybrid 32/21/87 had the lowest firmness of

the fruit, as opposed to hybrids IV/63/81 and 22/17/87 which had medium fruit firmness; at the same time, hybrids 38/62/70, IV/63/81 and 34/41/87, together with cultivar ‘Nada’ and standard ‘Čačanska Lepotica’ recorded the highest degree of this parameter.

Table 2. Fruit shape, skin colour, flesh colour and fruit firmness of plum hybrids.

Genotype	Fruit shape	Skin colour	Flesh colour	Fruit firmness
Hybrid 38/62/70	circular	violet blue	yellowish green	firm
Hybrid IV/63/81	elliptical	violet blue	yellowish green	medium
Hybrid 32/21/87	circular	dark blue	yellow	soft
‘Nada’	elliptical	dark blue	yellow	firm
Hybrid 34/41/87	elliptical	dark blue	yellowish green	firm
Hybrid 22/17/87	elliptical	violet blue	yellowish green	medium
‘Čačanska Lepotica’	elliptical	dark blue	yellowish green	firm

In the assessment of the fruit attractiveness, the primary importance is assigned to the fruit size, followed by its colour and shape. In certain Central European countries, preference is given to cultivars with an elongated fruit and blue colour of the skin, with intense bloom (Neumüller, 2010). The same author reports that firmness of the fruit and flesh colour represent highly important properties of table plum cultivars, where advantage is given to genotypes with firm fruits and orange of yellow flesh colour. In this regard, ‘Nada’ stands out owing to its elongated fruits with dark-blue skin and firm yellow flesh.

The soluble solids content and the content of total acids in the fruit, as well as the ratio between them were cultivar-specific for each of the examined plum genotype (Table 3).

Table 3. Soluble solids content, total acids content and soluble solids content and total acids ratio of plum hybrids.

Genotyp	Soluble solids content (%)	Total acids content (%)	Soluble solids content/ Total acids content
Hybrid 38/62/70	11.39±0.44 g	1.03±0.04 b	11.11±0.16 ef
Hybrid IV/63/81	12.62±0.65 f	1.06±0.02 b	11.98±0.80 e
Hybrid 32/21/87	14.27±0.52 d	1.42±0.05 a	10.25±0.58 f
‘Nada’	14.73±0.28 c	0.35±0.02 e	43.72±1.47 a
Hybrid 34/41/87	16.49±3.21 b	0.77±0.05 d	21.37±3.94 b
Hybrid 22/17/87	17.01±0.96 a	0.97±0.04 c	17.77±1.02 c
‘Čačanska Lepotica’	13.97±0.70 e	0.97±0.03 c	14.63±0.99 d

The different lower-case letters assigned to columns show significant differences for $P \leq 0.05$ after applying *LSD* test.

The highest average value of the soluble solids content was established in the fruits of hybrid 22/17/87 (17.01±0.96%), as the latest-ripening one, whereas the lowest value of this parameter was found in the fruits of hybrid 38/62/70 (11.39±0.44%) as the earliest-ripening one. Compared to ‘Čačanska Lepotica’ the highest content of soluble solids in the fruit was recorded in ‘Nada’ cultivar and hybrids 32/21/87, 34/41/87 and 22/17/87. The highest content of total acids in the fruit was found in hybrid 32/21/87 (1.42±0.05%), whereas the lowest value of this parameter was recorded in ‘Nada’ cultivar (0.35±0.02%). Compared to the standard, higher values of the total acids content were typical of the fruits of hybrids 38/62/70, IV/63/81 and 32/21/87. The highest ratio between the soluble solids content and the total acids content was established in ‘Nada’ cultivar (43.72±1.47), whereas the lowest value of this ratio was found in hybrid 32/21/87 (10.25±0.58). Compared to the control cultivar, the highest values of this parameter were typical of cultivar ‘Nada’ and hybrids 34/41/87 and 22/17/87 (Table 3).

The results obtained based on the analysis of the soluble solids contents in the fruits of the cultivars examined in this study confirm the existence of a positive correlation between the soluble solids content and the ripening time of the fruit (Neumüller, 2010). Considering the fact that the soluble solids content of ≥12,0% is generally taken as the limiting value for better acceptability of a plum cultivar by consumers (Crisosto *et al*., 2004), it can be concluded that these criteria are not met only by the earliest hybrid 38/62/70. Given the assumptions that later-ripening cultivars ought to have a soluble solids content in excess of 17% (Neumüller, 2010), hybrid 22/17/87 has made a distinction in this regard. With the exception of the values obtained for cultivar 'Nada', the total acids contents obtained in this paper were within the ranges reported in the studies conducted by Miletić and Petrović (1996). On the other side, Družič *et al*. (2007) have reported total acids content in certain German plum cultivars which corresponded to values obtained for cultivar 'Nada' in our research. It is a well-known fact that the ratio between the soluble solids content and total acids, rather than the very content of dry soluble solids, represents a reliable indicator of a cultivar's suitability for acceptance by consumers (Crisosto *et al*., 2004). It is also known that European plum cultivars are generally regarded as cultivars with a good fruit quality, provided that the ratio between the soluble solids content and the total acids falls within the range between 12 and 24 (Robertson *et al*., 1992). In this regard, hybrids 34/41/87 and 22/17/87 can be viewed as promising. Owing to its low content of total acids in the fruit, cultivar 'Nada' recorded considerably higher values of this parameter compared to the upper border value reported by the cited author. Similar information is reported by Družič *et al*. (2007) for 'Elena' cultivar.

Conclusion

The late ripening time and the large size of the fruit, its firmness, elongated shape, dark blue colour of the skin, the yellow flesh and good chemical composition – these are all properties that support the assertion that the new cultivar 'Nada' represents a genotype with the best combination of positive characteristics. Considering its early ripening time, as well as the large size and firmness of its fruits, hybrid 38/62/70 can also be interesting, and so can be hybrid 22/17/87, from the point of view of its late ripening time and the good chemical composition of the fruit. Apart from their obvious potential in production, the examined cultivars can be used in future plum breeding programmes.

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EFFECTS OF SUBSURFACE DRIP IRRIGATION ON PRODUCTIVE CHARACTERISTICS OF SEVEN POTATO CULTIVARS

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Abstract

In the total food production in Serbia, potato (*Solanum tuberosum* L.) occupies an important place. The great economic importance of potato arises from the fact that this crop is grown on 78.000 ha, with an average yield of 11.3 t ha^{-1}. The average yield in Serbia is significantly lower compared to average potato yield in Europe of 37.0-55.0 t ha^{-1}. Commercial production of potato is carried out in the 50.000-60.000 ha with an average yield of about 15-25 t ha^{-1}, but it still does not nearly meet the standards of modern agricultural production. Every 3-4 years, the major problem in potato production in our country is extreme drought that limits crop yield and quality of tubers. A two-year study (2011 and 2012) was conducted to investigate the effects of subsurface drip irrigation on productive characteristics of different potato cultivars: Desiree, Marabel, Laura, Agria, Bellarosa, Carrera and Jelly. Field trials were carried out on a slightly calcareous chernozem soil at Zemun Polje and experimental design was two factorial split plot with four replications. The occurrence of stress during the growing season, as a result of high soil temperature, lack of soil moisture, compaction and poor aeration resulted in significant reduction in potato productivity. Subsurface drip irrigation significantly increased mass of tubers and total tuber yield in both 2011 and 2012, while the number of tubers per plant was mainly determined by genotype/cultivar.

Key words: *drip irrigation, potato yield, drought*

Introduction

Potato yield is influenced by numerous factors during growing season, such as selected cultivar, planting time, agricultural practice and weather conditions. Soil water shortage is a common adverse factor for the growth of plants in the field. Potato is the crop grown in the moderately moist climate and drought has negative impact on all yield components. One of the reasons for potato sensitivity on soil water deficit is shallow root system whose base mass is concentrated in upper soil layer approximately at 0.3 m. Some authors consider drought to be the main yield-limiting factor in the global potato production (Van der Zaag & Burton 1978). Long period of intensive drought stress could retard the growth of plants, causing the changes of the morphological structure and the distribution pattern of biomass, or even death (Christina and Gisela, 2013; Dias et al., 2007).

Sensitivity of the potato crop to water stress varies with the developmental stage of the crop. During first stages of vegetative growth, potato requires minimally 70% field water capacity (FWC), while requirements at the flowering and tuber-bulking stages are 70-80% FWC. Water deficit during tuber initiation and onset of tuber growth significantly decreases yield and increases portion of deformed tubers, while at tuber bulking stage significantly reduces tuber quality and yield (King & Stark, 2011; Poštić et al., 2013). Abiotic stress conditions, such as high soil temperatures, low soil moisture, soil compactness and low

aeration, cause secondary growth of tubers and thus lower market value of potato (Beukema and Van der Zaag, 1979; Momirović et al., 2000).

In the last several years, serious problem in potato production in Serbia is occurrence of extremely dry periods that limit crop production (Broćić et al., 2009; Poštić et al., 2012). Irrigation is an important agrotechnical practice for reduction of drought effects, which has a key role in attaining high and stable yields. In arid regions, potato yield linearly accedes with addition of water amount that, altogether with precipitations, compensate amount lost by evaporation (Ferreira and Goncalves, 2007; Bošnjak et al., 1996). During hot and dry summers, potato can lose over 50 mm H_2O per week by evaporation and irrigation represents prerequisite for secured production (Bošnjak, 1994). Subsurface drip irrigation ensures optimal water supply in hilled potato rows, where tubers are form, during whole vegetative period. The major objective of this study was to investigate the effects of subsurface drip irrigation on productive characteristics of seven potato cultivars: Desiree, Marabel, Laura, Agria, Bellarosa, Carrera and Jelly.

Materials and methods

Virus-free tubers of seven commercial potato cultivars, Desiree, Marabel, Laura, Agria, Bellarosa, Carrera and Jelly, were obtained from Solanum Komerc (Guča, Serbia). Tubers were planted in the experimental field in Zemun Polje, Serbia (44°88′ N, 20°35′ E, 82 m altitude) by early April in years 2011 and 2012. Two-factorial split plot design with four replications was used and plots of 21 m^2 with 5 rows spaced 80 cm apart were seeded. Shortly before planting, tubes for subsurface drip irrigation were positioned and buried in the middle of hilled rows at 0.15 m depth. Soil was irrigated to continuously maintain 75-80% FWC. Control plots received no special treatment and they were watered only by natural rainfall. The soil type was slightly calcareous chernozem with high clay content (>32%). Calcium carbonate content was 3.89-4.54% in the humus-accumulative horizon, which determines soil as slightly calcareous. Soil solution was neutral to slightly alkaline (pH: in water – 7.60-7.75, in nKCI 6.75-6.90), which was adequate for potato growing. According to humus content, soil was medium fertile, with high amount of P_2O_5 (28.55-31.00 mg per 100 g of soil) and K_2O (29.10-34.80 mg per 100 g of soil). Investigated slightly calcareous chernozem belongs to soils with a medium productivity potential, requiring irrigation, besides fertilization, for successful crop production during the dry years.

Standard tillage treatment for potato crop was used. Pre-sowing soil treatment included fertilization with Multi-Comp base and BitterMag, as well as organic fertilizer Stallatiko. Plants were foliar fertilized with Haifa Bonus (N:P:K = 13:2:44, 6 kg ha-1), Haifa Chemicals, Israel. Weeds and pests were controlled post-emergence with commercial formulations of Tarot 25 WG (rimsulfuron 12.5 g ha-1), Du Pont International, Switzerland, Force 0.5 G (tefluthrin 75 g ha^{-1}), Syngenta, Switzerland, Actara 25 WG (thiamethoxam 15 g ha^{-1}), Syngenta, Switzerland.

Data considering basic climate parameters (daily maximum and minimum air temperature, precipitation quantity and distribution) were collected in the period April-September. At the end of vegetation period, 8 samples, each comprising tubers of plants developed from common seed, were collected per each investigated cultivar. Tuber number and mass, as well as yield per plot were analyzed. Total yield per ha was estimated theoretically according to plant density in the field.

Table 1. The average monthly air temperatures and precipitation from April to September.

Months	Temperature (°C)			Precipitation (mm)		
	2011	2012	1981-2010	2011	2012	1981-2010
April	14.5	14.6	12.4	14.1	66.9	56.8
May	17.7	18.0	17.2	66.8	127.9	58.0
June	22.1	24.6	20.1	41.4	16.0	101.2
July	24.2	26.9	21.8	95.0	39.0	63.0
August	24.6	26.0	21.4	14.0	4.5	58.3
September	22.9	22.0	17.7	47.7	30.7	55.3
Average/Sum	**21.0**	**22.0**	**18.4**	**279**	**285**	**392.6**

Weather conditions during potato vegetation period were unfavorable, especially in year 2012 which was extremely hot and dry. During summers 2011 and 2012, average monthly air temperatures were significantly higher than multiple-year average (1981-2010), which adversely affected potato growth and development. Precipitations in summers 2011 and 2012 were below limits of multiple-year average with an uneven distribution during vegetative period in both years.

Statistical analysis was performed using STATISTICA 10 (StatSoft, Inc. 1984-2011, USA). The data concerning tuber number, tuber mass or yield were subjected to two-factor analysis of variance (ANOVA) with Irrigation and Cultivar as factors. If significant interaction between factors was determined, the means were separated by Fisher's-LSD multiple range test at the significance level $\alpha = 0.05$ for each Cultivar and Irrigation combination. Otherwise, LSD multiple range test (significance level $\alpha = 0.05$) was conducted for one significant factor at a time, Cultivar or Irrigation, but on each level of the other factor.

Results and discussion

Data regarding productive characteristics of seven investigated potato cultivars are presented in Tables 2, 3 and 4. In general, investigated parameters/productive characteristics mainly had higher values in 2011 compared with 2012. The probable reasons for observed differences are lower average air temperatures and less uneven distribution of precipitations during potato vegetation period in year 2011 (Table 1.).

The number of tubers per plant was mainly determined by genotype/cultivar, while irrigation increased number of formed tubers only in cvs. Agria and Carrera in extremely hot 2012. Conversely, mass of tubers was mainly affected by irrigation. In year 2012, drip-irrigated potato cultivars, with exception of cv. Desiree, produced from 1.4- to 2.2–fold heavier tubers compared to variant without irrigation. Total yield was also affected by irrigation in years 2011 and 2012. Interestingly, yield of only two cultivars, Agria and Carrera, significantly increased with irrigation in moderately hot 2011 and irrigated Carrera had the highest yield (68.3 t ha^{-1}) in this particular year. In extremely hot and dry year 2012, the lowest yield was determined for cv. Agria (11.4 t ha^{-1}) without irrigation. Irrigation was an especially significant factor in this year, which caused from 1.4- to 2.7-fold increase in yield of seven investigated cultivars. Drip-irrigated Laura had the highest yield (45.2 t ha^{-1}) in 2012, while irrigated Carrera and Agria showed the highest increase in productivity (2.5- and 2.7-fold increase) compared to non-irrigated control.

Table 2. Effects of subsurface drip irrigation on the number of tubers in 2011 and 2012

Cultivar	2011		2012	
	Without irrigation	Irrigation	Without irrigation	Irrigation
DESIREE	8.9±1.6 ^{abc}	7.8±1.1 ^{ab}	^{1} 8.0±1.1 ^{a}	^{1} 9.5±1.3 ^{ab}
MARABEL	11.6±1.7 ^{bc}	11.3±2.1 ^{c}	^{1}11.9±0.5 ^{b}	^{1} 10.4±0.4 ^{a}
LAURA	7.6±0.8 ^{a}	10.4±0.6 ^{bc}	^{1}12.0±0.5 ^{b}	^{1} 13.3±0.8 ^{c}
AGRIA	8.0±2.0 ^{ab}	7.9±0.2 ^{ab}	^{1} 6.8±0.8 ^{a}	^{2} 8.9±0.4 ^{ab}
BELLAROSA	7.4±0.7 ^{a}	6.1±0.7 ^{a}	^{1} 6.5±0.5 ^{a}	^{1} 7.3±0.7 ^{b}
CARRERA	7.4±0.2 ^{a}	9.3±0.9 ^{abc}	^{1} 6.1±0.7 ^{a}	^{2} 9.5±0.9 ^{ab}
JELLY	12.0±1.0 ^{c}	10.1±0.6 ^{bc}	^{1}10.9±0.6 ^{b}	^{1}10.1±1.7 ^{a}
Two-factor ANOVA				
Cultivar (A)	**		***	
Irrigation (B)			*	
A x B				

Significant differences (α=0.05) among Irrigation treatments are indicated by different numbers in each row. Significant differences (α=0.05) among Cultivar are indicated by different letters in each column. Results of two-factor ANOVA are indicated by asterisks (*, p<0.05; **, p<0.01; ***, p<0.001).

Table 3. Effects of subsurface drip irrigation on tuber mass (g) in 2011 and 2012

Cultivar	2011		2012	
	Without irrigation	Irrigation	Without irrigation	Irrigation
DESIREE	^{1} 88.4±7.9	^{2}163.7±17.6 ^{ab}	^{1} 52.1±7.3 ^{abc}	^{1} 73.8 ±9.5 ^{a}
MARABEL	^{1} 80.5±9.5	^{2}118.6±9.0	^{1} 58.1±0.9 ^{bc}	^{2} 92.1±6.0 ^{a}
LAURA	^{1}104.2±32.0	^{1}126.2±6.5	^{1} 37.9±5.1 ^{a}	^{2} 82.6±5.9 ^{a}
AGRIA	^{1}112.4±10.9 ^{ab}	^{2}172.9±20.1 ^{ab}	^{1} 42.0±5.8 ^{ab}	^{2} 83.7 ±5.4 ^{a}
BELLAROSA	^{1}116.8±15.5	^{1}157.2±44.0	^{1} 74.9±1.9 ^{d}	^{2} 127.6± 6.9 ^{b}
CARRERA	^{1}117.8±14.4	^{2}179.7±10.3 ^{abc}	^{1} 60.1±7.8 ^{cd}	^{2} 95.9 ±10.3 ^{a}
JELLY	^{1} 98.1±10.6	^{1}139.7±14.5	^{1} 43.6±6.9 ^{abc}	^{2} 83.1±12.2 ^{a}
Two-factor ANOVA				
Cultivar (A)			***	
Irrigation (B)	***		***	
A x B				

Significant differences (α=0.05) among Irrigation treatments are indicated by different numbers in each row. Significant differences (α=0.05) among Cultivars are indicated by different letters in each column. Results of two-factor ANOVA are indicated by asterisks (*, p<0.05; **, p<0.01; ***, p<0.001).

Table 4. Effects of subsurface drip irrigation on total yield of potato (t ha^{-1}) in 2011 and 2012

Cultivar	2011		2012	
	Without irrigation	Irrigation	Without irrigation	Irrigation
DESIREE	^{1} 33.9±8.1	^{1} 51.4±5.9	16.6±2.0 ^{AB}	28.0±1.9 ^{CD}
MARABEL	^{1} 38.1±5.7	^{1} 53.8±7.5	28.7±1.0 ^{D}	39.8±3.2 ^{FG}
LAURA	^{1} 35.5±14.6	^{1} 54.7±5.2	18.7±1.9 ^{AB}	45.2±2.4 ^{G}
AGRIA	^{1} 35.1±5.5	^{2} 56.4±5.9	11.4±0.7 ^{A}	30.7±1.1 ^{DE}
BELLAROSA	^{1} 35.0±3.2	^{1} 38.1±8.3	20.3±1.7 ^{BC}	38.4±4.0 ^{EFG}
CARRERA	^{1} 35.6±4.8	^{2} 68.3±4.4	15.2±2.2 ^{AB}	38.0±5.7 ^{EFG}
JELLY	^{1} 49.7±8.4	^{1} 57.9±3.4	19.6±3.1 ^{B}	32.6±2.7 ^{DEF}

Two-factor ANOVA		
Cultivar (A)		***
Irrigation (B)	***	***
A x B		*

Significant differences (α=0.05) among Irrigation treatments are indicated by different numbers in each row. Significant differences (α=0.05) among Irrigation X Cultivar treatments are indicated by different capital letters. Results of two-factor ANOVA are indicated by asterisks (*, p<0.05; **, p<0.01; ***, p<0.001).

Conclusions

Results of our study, conducted in moderately hot 2011 and extremely hot and dry 2012 on seven potato cultivars, indicate that the number of tubers per plant was mainly determined by genotype/cultivar, while irrigation affected mass of tubers and total tuber yield. Two of the cultivars, Agria and Carrera, responded exceptionally well on subsurface irrigation in both investigated years. Drip-irrigated cv. Carrera had the highest yield (68.3 t ha^{-1}) in moderately hot 2011, while irrigated cv. Laura had the best yield (45.2 t ha^{-1}) in extremely hot and dry year 2012. Marabel was the most productive cultivar without irrigation with yield of 28.7 t ha^{-1} in year 2012.

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EFFECT OF NITROGEN FERTILIZERS ON LEAF BIOMASS PRODUCTION OF ENERGY CROPS (MISCANTHUS X GIGANTEUS)

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Abstract

Being under great pressure to improve energy safety in terms of environmental protection and to decrease dependence on imports, many countries shift their production to alternative bio-fuels, such as ethanol and bio-diesel obtained from plant products.

The subject-matter of the study is two-year research (2012-2013) on variability of morphological properties of Miscanthus depending on nitrogen rates used for fertilization. The study analysed the following morphological properties: plant height, number of leaves and leaf width. The results show that increased nitrogen rates significantly affect the investigated morphological properties. In fertilization variants, plants were significantly higher than in the control variant in both years of research, which affected the number of formed leaves and leaf width. The effect of the applied nitrogen rates depended on weather conditions (distribution of precipitation). In a favourable precipitation regime the optimal biomass growth was obtained when 80 kg ha^{-1} nitrogen was applied.

Having analysed the correlations, the authors found strong statistical dependence among the investigated parameters. A non-significant positive correlation was found between plant height and precipitation and between number and width of leaves and precipitation. A non-significant negative correlation was found between plant height and temperature of air, as well as between number and width of leaves and temperature of air.

Key words: *agro-ecological conditions, nitrogen, biomass, Miscanthus, morphological properties*

Introduction

Miscanthus is a perennial, highly productive C_4-grass, originating from eastern Asia. It belongs to so-called “bioenergy crops”, whose stem is dried and used as a fuel, thus being an alternative to existing energy sources. According to the results of Lewandowski et al. (2010), the energy value of Micanthus biomass is at the level of firewood (17.7 MJ kg^{-1}). Considering these data, the most of developed countries have set a goal to increase crop production suitable for energy production from renewable energy resources. A strong incentive has been given to this research after the adoption of the *Kyoto Protocol* (1997) on climate change and reducing greenhouse gas emissions and the greenhouse effect (Glamočlija et al., *2007*).

Vegetative growth begins when shoots emerge from underground stems-rhizomes in early April. Annual above-ground stems grow from the intercalary meristems. The stems are nodded, straight and unbranched. They reach their maximum height in late August. At the end of vegetative growth, tassels with sterile flowers emerge, and assimilates start to move down to the underground stems-rhizomes. Plants use these nutrients to form new stems next year. Over years, rhizomes branch, increasing their mass. In commercial production, there are used as planting material for new Miscanthus plantations (Dželetović et al., 2009).

Miscanthus growing requires a very simple technology, what can be conducted by using standard machinery. Only for harvest it is necessary to use some specific machinery – combines that work on the principle of silo–combines, compressing harvested plants in bundles or bales, depending on the future use of this biomass. According to numerous authors, the best time to harvest Micanthus is when there is a minimum amount of water in stems. The stems harvested in this period will have the highest energy value, as pointed out by Dželetović (2002), Lewandowski et al. (2010) and other researchers. Previous studies have shown that the Miscanthus stem has the minimum amount of water during the winter period (January, February). However, snowfall can damage some of the above-ground biomass, which affects yields (Dželetović et al., 2012). In areas without heavy snowfall *Lewandowski et al*. (2010) recommend having a so–called "late harvest" at the end of winter when stems have the minimum amount of water. Yields, however, may be up to 30% lower than usual.

Previous studies on Miscanthus crop practice in the first year of planting have shown that the limiting factor to successful Miscanthus production is weediness, especially if it is planted on weedy and deserted fields (Dražić et al., 2010; Ikanović et al., 2010). Perennial grasses predominate on such soil, making it difficult to use herbicides against narrow-leaved weeds. The same authors emphasize that using total herbicides is not efficient enough since most grassy weeds appear later, at the time of shoots emerging.

The main objective of this paper is to introduce energy crops in our agricultural production, in order to determine the adaptability of these plant species to agro-ecological and soil conditions in our production area, characterized by continental climate with cold winters, a small amount of snowfall and very hot and dry summers. Miscanthus environmental requirements are quite specific, since it is a perennial plant with rhizomes that over winter in a shallow soil layer. On the other hand, Miscanthus is tolerant to short–term drought, but it also very sensitive to the lack of precipitation in the year of planting (Dražić et al., 2014).

Material and methods

The research was conducted in 2012 and 2013, on brown forest soil in Sremska Mitrovica, Serbia. A multi-year trial was set up in a randomized block design with three replications. The subject-matter of the research was Miscanthus. Miscanthus rhizomes, purchased from a commercial supplier from Austria, were manually planted in the pre-prepared soil. Planting was carried out using 10-15 cm long rhizomes with 2-5 nodes, at the density of 20,000 rhizomes per ha^{-1}. Planting was conducted in the first decade of April, to the depth of 10 cm.

The research comprised two variants with applied nitrogen (N_1=50 kg ha^{-1} and N_2=80 kg ha^{-1}). A variant without nitrogen was taken as a control variant (N_0). Nitrogen was applied into soil during planting preparations in the first year, and again when shoots emerged in the second year.

Soil and meteorological conditions

The field trial was set up on brown forest soil (*Eutric Cambisol*, according to the FAO soil classification system). This type of soil has low levels of humus (1.38%) and total nitrogen (0.114%). It is poor in easy available phosphorus (5 mg per 100 g soil) and potassium (11.8 mg in 100g soil). Due to an extremely acid reaction (pH 6.1 in H_2O), it has a high content of available Fe, Cu, Mn and Zn, as well as Pb derived from the rock it was formed on (Pb-Zn belt on the bottom of Mt Avala and Mt Kosmaj (Glamočlija et al., 2012, 2015).

Meteorological conditions (temperatures and precipitation) in a particular area can be quite variable, so it is necessary to monitor them during the growing season, and to know their effect on physiological processes and critical stages of plant development, in order to achieve the maximum productivity of crops (Popović, 2010; Ikanović et al., 2015a, 2015b).

Meteorological data were retrieved from the weather station in Sremska Mitrovica. The average monthly air temperature in the 2012 growing season was 11.79 ^{o}C, which is 0.29^{o}C

higher than in 2013 (11.50 ^{o}C). Total amount of precipitation in 2013 was 809 mm, being 34 mm higher than in 2012 (775 mm) (Graph 1). The thermal regime in the growing season is very important for the optimal development of Miscanthus. In the year of planting, overwintering depends on the planting depth. Shallow planted Miscanthus with poorly developed rhizomes is particularly sensitive and such rhizomes often suffer from frost and large amounts of precipitation in winter (Dželetović et al., 2000).

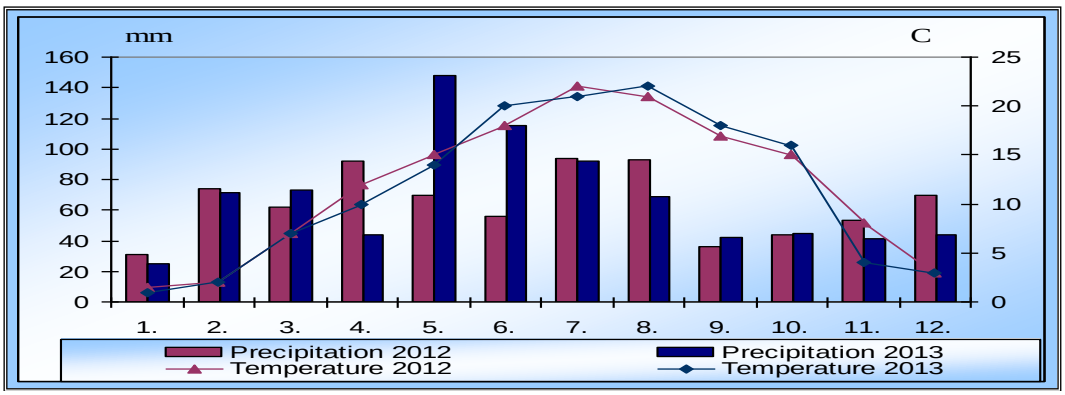


Fig. 1. Precipitation (mm) and temperatures (ºC), Sremska Mitrovica, Serbia, 2012-2013

Meteorological conditions, especially the water regime in the year of planting, were not favorable due to the shortage of precipitation in April and May, which resulted in slow emergence of plants. Year 2013 was more favorable for Miscanthus. Miscanthus has quite specific environmental requirements, since it is a perennial plant with perennating roots and rhizomes that overwinter in a shallow soil layer. On the other hand, Miscanthus is tolerant to short-term drought but it also very sensitive to the lack of precipitation in the year of planting.

Results and discussion

Miscanthus morphological properties

Miscanthus is an ideal energy crop, considering its high multi-year production of dry biomass, efficient utilization of nitrogen and water, good resistance to drought and the possibility of growing on degraded soil (Ikanović i sar., 2015a).

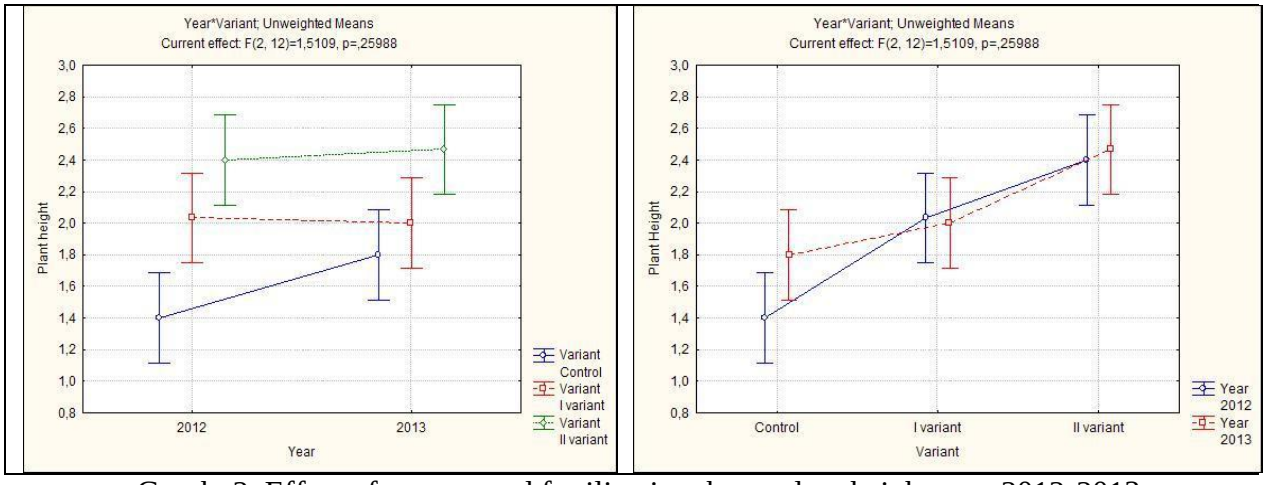
Plant height

This parameter was significantly affected by the applied nitrogen rates (Table 1). The average plant height was 2.02 m. The lowest values were recorded for the control variant, and highest in the variant with 80 kg ha^{-1} nitrogen. The variants with nitrogen and the interaction between the factors, as well as variant x year interaction had great effect on plant height ($p<0.5$). On average, the plants in 2012 were lower than in 2013, but this difference was not significant (Graph 2.a, 2.b). Standard deviation for plant height averaged 0.416 (Table 1).

Table 1. Miscanthus morphological properties, 2012-2013

Parameter	Control	Variant 1	Variant 2	Average	Std. Dev.	Std. Err
Plant height, m						
2012	1.40	2.03	2.40	1.94	0.45	0.15
2013	1.80	2.00	2.47	2.09	0.39	0.13
Average	1.60	2.02	2.43	2.02	0.41	0.09
Number of leaves						
2012	9.00	11.67	16.33	12.33	3.50	1.16
2013	8.67	13.66	16.00	12.78	3.45	1.15
Average	8.83	12.66	16.16	12.55	3.38	0.79
Leaf width, cm						
2012	1.06	1.47	1.83	1.45	0.36	0.12
2013	1.17	1.80	1.93	1.63	1.40	0.13
Average	1.12	1.63	1.88	1.54	0.38	0.09

Parameter	LSD Test	Year	Variant	Y X V
Plant height	0.5	0.233	0.286	0.404
	0.1	0.328	0.402	1.751
Number of leaves	0.5	1.538	1.883	2.663
	0.1	2.165	2.651	3.749
Leaf width	0.5	0.200	0.245	0.347
	0.1	0.282	0.346	0.488



Graph. 2. Effect of year, a, and fertilization, b, on plant height, cm, 2012-2013

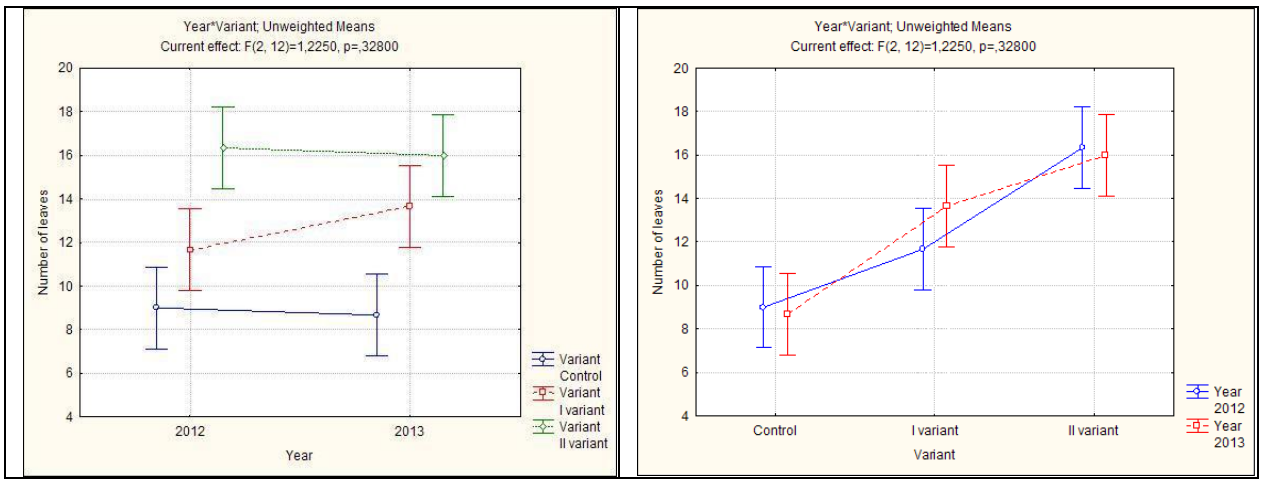
Meteorological conditions during the growing season affected plant height. In their research, Ikanović et al. (2015a) reported that the weather conditions had a significant effect on morphological and productive properties of Miscanthus. Dražić et al. (2014) point out that Miscanthus has not been put yet in commercial production, although the experimental results show that it can be successfully grown in Serbian agro–ecological and soil conditions.

Number of leaves

The average number of leaves per stem was 12.55. When observed by years, the plants in 2012 had less leaves on average than in 2013, but this difference was not significant (Graph 3.a). Standard deviation for plant height averaged 3.38 (Table 1).

With an increase in the amount of nitrogen the number of leaves also increased, statistically being significantly higher in the variant with the maximum nitrogen rate than in the control

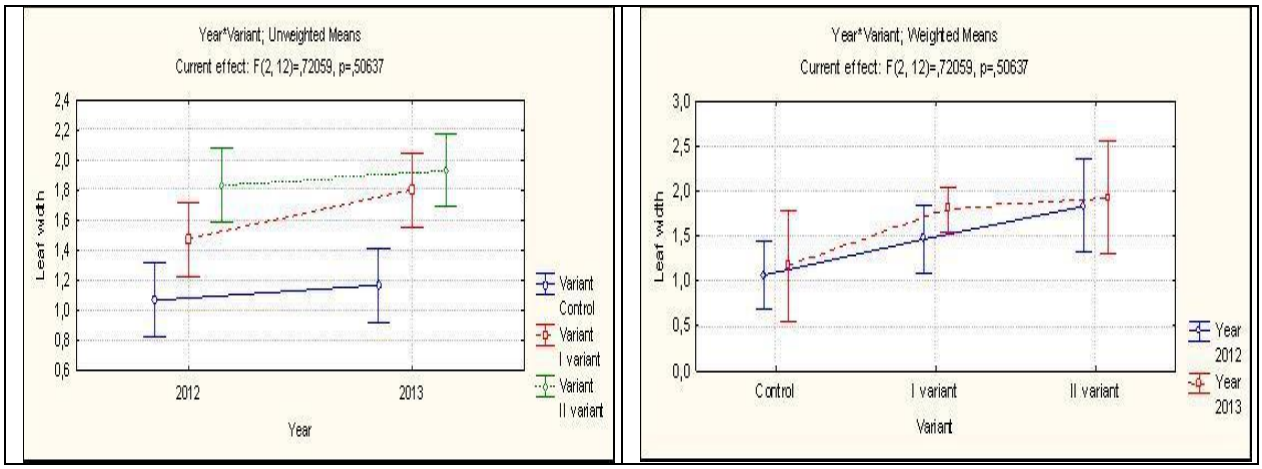
variant. On average, plants had a significantly higher number of leaves in variant 1 with 50 kg ha^{-1} (N_1) and in variant 2 with 80 kg ha^{-1} (N_2) than in the control variant (N_0) (Table 1 and Graph 3.b).Variant and the interaction of factors, as well as variant and years of research exhibited statistically significant effect on the number of leaves ($p<0.5$).



Graph 3. Effect of yield, a, and fertilization, b, on the number of leaves, 2012-2013

Leaf width

The average leaf width was 1.45 cm, Table 1, Graph 4a, 4b.



Graph 4. Effect of yield, a, and fertilization, b, on leaf width, 2012-2013

On average, lower values of this property were recorded in 2012 than in 2013, but the difference is not significant (Graph 4.a). Like in the previous parameters, variant and interaction, and variant and year of research exhibited statistically significant effect on the number of leaves ($p<0.5$). On average, plants had significantly higher values of this property in the variants with nitrogen than in the control variant (Table 1, Graph 4.b). Standard deviation for plant height averaged 0.38 (Tab. 1).

Interdependence of the investigated properties

The research studied correlations (interdependence) between the investigated properties. A significant and strong positive correlation was found between plant height and the number of leaves, whilst a significant positive correlation was recorded between plant height and leaf width (Table 2). The coefficient of correlation indicates that the number of leaves per stem and plant height are positively and statistically very strongly correlated.

Table 2. Coefficients of correlations between the investigated properties

Parameters	Plant height	Number of leaves	Leaf width	Temperature	Precipitation
Plant height	1.00	0.76**	0.64*	-0.18^{ns}	0.20^{ns}
Number of leaves	0.76**	1.00	0.84**	-0.07^{ns}	0.10^{ns}
Leaf width	0.64*	0.84**	1.00	-0.24^{ns}	0.20^{ns}
^{ns} –non significant; * significant at p < 0.5 and ** significant at p < 0.1					

The authors recorded a highly significant positive correlation between the number of leaves and leaf width, as well as a non-significant positive correlation between plant height and precipitation and the number of leaves, leaf width and precipitation (Table 2).

Plant height and temperature were non-significantly and negatively correlated, as well as the number of leaves, leaf width and temperature (Table 2). The results of previous research on the effect of increased nitrogen rates on C_4 grasses confirm these plants are greatly depended on nitrogen, which was shown as a statistically significant increase of leaf mass in total above-ground biomass (Ikanović et al., 2010; Glamoclija et al., 2007). When water regime is favorable, shoots emergence is better in the first year and Miscanthus has larger leaf mass and a greater number of leaves, as confirmed by the results of Ercoli et al. (1999).

Živanović et al. (2014) and Dražić et al. (2014) point out a manifold significance of energy crops, which is reflected in a more rational utilization of soil less favorable for production and soil under recultivation. The same authors also point out another advantage – growing energy crops in order to obtain more biomass and energy than from post-harvest residues. Moreover, a significant improvement of the ecosystem was noticed, since these crops have intensive annual growth of biomass and use large amounts of carbon dioxide and other gases from the atmosphere.

Conclusions

The following conclusions were made from the research:

During the two-year research, the average plant height ranged from 1.94 m to 2.09 m. The average values of plant height were higher in 2013 than in 2012. The nitrogen variants had significantly higher plants than the control variant. The average number of leaves was 12.55 and varied from 12.33 to 12.78 (in 2012 and in 2013). Significantly higher number of leaves was recorded in the variants with nitrogen than in the control variant. The average leaf width was 1.54 cm. This parameter had higher average values in 2013 than in 2012. In the variants with nitrogen, plants were significantly higher than in the control variant.

Having analyzed the correlations, the authors recorded a statistically strong positive relationship between the investigated Miscanthus properties.

A non-significant positive correlation was found between plant height and precipitation, as well as between the number of leaves, leaf width and precipitation, whilst a non-significant negative correlation was found between plant height and temperature, the number of leaves, leaf width and temperature.

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CHROMIUM (Cr) CONTENT IN THE ABOVEGROUND PORTIONS AND KERNEL OF DIFFERENT SMALL GRAIN CULTIVARS

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Abstract

To ensure safe food production, cultivar-specific traits of winter small grains were analysed in terms of mineral nutrition in order to determine differences between small grain (barley, oat and triticale) genotypes in the level of certain heavy metals (Ni). This study examined the effect of mineral (NPK), organic (manure) and lime (Njival Ca) fertilisers on the uptake of chrome and its content in kernel and the aboveground portions of winter small grains. The investigation was conducted under field conditions in a soil having acid reaction (pH_{KCl} = 4.20) it is included of two winter barley cultivars, one winter oat cultivar and one winter triticale cultivar. Apart from the control, four various fertilisation variants were also included in the trial: NPK; N +$CaCO_3$; NPK + $CaCO_3$; NPK + $CaCO_3$, + organic (manure). The kernel of mature small grain plants and their vegetative biomass were found to contain quite different levels of Cr, depending on genotype properties and fertilisation systems. The amount of chromium in the above-ground portions was higher than the kernel, and that relationship is as follows: Control -3.70; NPK - 4.72; N + $CaCO_3$ - 1.79; NPK $CaCO_3$ + - 2.5; NPK + $CaCO_3$ + organic manure -4.8. On the $CaCO_3$ + NPK + manure of his mobility is lowest, because the application of manure reduces the mobility of chromium (DTPA-extraction) and thus reduces its toxic effects in plants.

Key words: *cultivar, fertilisation, chromium, small grains*

Introduction

Heavy metals in soils originate primarily from geochemical sources i.e. from the lithosphere, and their concentration in soils is dependent on their content in the parent rock (Ubavić et al., 1995). The behaviour of heavy metals in soils is governed by a range of factors that affect their mobility and accumulation by plants, the most important being soil reaction, organic matter content and colloidal clay content (Pelivanoska, 2011). In addition to these factors, the mobility and harmful effects of heavy metals can also be induced by moisture, calcium carbonate content, hydrated iron and aluminium oxides, cation exchange capacity, redox potential, groundwater level etc. (Pelivanoska, 2011).

Recent research has also identified the increasing presence of heavy metals in agricultural soils (Markoski et al., 2011) which are further endangered by the increasing or inappropriate use of chemicals, as well as by wastewaters, sewage, sludges and mineral fertilisers. Polluted soils are an important factor contributing to total chromium concentrations in plant tissues. Plants growing on soils highly contaminated with chromium contain higher levels of chromium compared to similar plants on soils with a low content of this element (Cary and Kubota, 1990).

Similar to arsenic, chromium is a redox active soil contaminant but one that has dramatic alterations in toxicity and mobility with changes in oxidation state. Trivalent Cr is rather benign to most plants and animals and binds strongly to soil solids (Fendorf, 1995). In contrast, hexavalent Cr is toxic to living cells, being a Class A human carcinogen (Kargacin et al., 1993); because of its anionic nature and inability to form strong chemical complexes with

most soil materials, chromate is also highly mobile within the surface environment (Fendorf, 1995; Ball and Nordstrom, 1998).
Most toxic elements typically react with organic compounds to produce stable complexes with ligands containing oxygen, sulphur or nitrogen as electron donors. The toxic effect is based on their irreversible binding to metabolically active groups in amino acids, polypeptides and proteins (Mihaljev et al., 2008). It is understood that toxic elements primarily affect the cell membrane, whereas their secondary effect in most cases includes damage to the enzyme systems residing in the cell interior (Milošević and Vitorović, 1992). Through plants, heavy metals enter the food chain and have cumulative properties in the human body i.e. they accumulate in certain organs or tissues, where they exhibit their deleterious effect.
Small grains (oat, barley and triticale) are annual plants considered the leading and major field forage crops for quality feed production, and are also used fresh and preserved as hay, silage and flour.
The objective of this study was to evaluate the content of Cr in the aboveground vegetative biomass and kernel of small grains to obtain information on the safety of these feedstuffs. Cultivar specificity of winter small grains with respect to mineral nutrition was analysed to determine differences among genotypes in the content of certain heavy metals (Cr).

Materials and methods

The investigations were conducted over a three–years period (1999/00–2001/02) at an experimental field of the Small Grains Research Centre in Kragujevac, on the very acid soil (pH in KCl 4.20). The trial was set up according to a randomized block design in three replications. The trial plot was 14 m^2 (7 m x 2 m). The trial involved an examination two barley cultivars (Jagodinac and Grand), one oat cultivar (Vranac) and one triticale cultivar (Knjaz). The following fertilization treatments were used in the trial: 1. the control (without fertilization); 2. NPK; 3. N + CaCO3; 4. NPK + CaCO3; 5. NPK + manure + CaCO3. The mineral fertilizer was used at the rate of 500 kg/ha NPK (15:15:15), nitrogen at the rate of 75 kg N ha^{-1} (as KAN), and lime (CaCO3) at the rate of 2.0 t ha^{-1}. "Njival Ca", the product of the Serbian Glass Factory–Paracin, was used as a lime fertilizer with 98.5% CaCO3. The applied farmyard manure was a mixture of poultry and cow manures, and it was well–matured (maturing for several years). Kernel samples were collected at the full maturity stage and the aerial plant parts were sampled at the heading/tasselling stage. Cr and Cd concentrations were determined by the AAS method following dry sample burning.

Results and discussion

Chromium is an essential element for animals, given its vital role in normal carbohydrate and lipid metabolism. In humans, there is an increased need for chromium in people with impaired glucose tolerance. The essential role of Cr in plant metabolic processes is not known, but in some plant species low concentrations of certain non-essential heavy metals (Cr) can stimulate plant growth and development (Ernst, 1996). In agricultural soils, Cr originates not only from the parent material, but also from sludge, composted waste and fungicides, which are increasingly used. Similar to arsenic, chromium is a redox active soil contaminant but one that has dramatic alterations in toxicity and mobility with changes in oxidation state. Trivalent Cr is rather benign to most plants and animals and binds strongly to soil solids (Fendorf, 1995). In contrast, hexavalent Cr is toxic to living cells, being a Class A human carcinogen (Kargacin et al., 1993); because of its anionic nature and inability to form strong chemical complexes with most soil materials, chromate is also highly mobile within the surface environment (Fendorf, 1995; Ball and Nordstrom, 1998). Chromium forms very strong bonds in soils rich in clay and organic matter, and therefore has very low mobility and is adsorbed in the 5-10 cm deep topsoil. Cr amounts in the soil range from 5 to 100 mg/kg (Ubavic and

Bogdanovic, 1995), but they can often occur at much higher concentrations, up to 3400 mg/kg (Markoski et al., 2001), particularly in soils formed on serpentine rocks. The concentrations of available Cr in soils are very low, hence its very low content in plants of 0.2 to 0.4 mg/kg (Pelivanoska et al., 2001).

Table 1. *Cr content in of the small grain plants (mg kg^{-1})*

Fertilization variants												
	A_1	A_2	A_3	A_4	A_5	x B	A_1	A_2	A_3	A_4	A_5	x B
Cultivars	*Small grains – above ground portions*						*Small grain – kernel*					
j B1	2.0	2.2	1.5	0.5	0.8	1.4	0.33	0.13	0.53	0.15	0.10	0.33
jB2	1.2	1.3	1.0	1.2	1.0	1.1	0.13	0.13	0.65	0.25	0.20	0.27
oB3	0.4	0.6	0.5	0.5	0.5	0.5	0.30	0.40	0.40	0.38	0.15	0.32
tB4	0.4	0.6	0.5	0.4	0.6	0.5	0.33	0.35	0.38	0.28	0.15	0.27
xB	1.0	1.18	0.88	0.65	0.72	0.9	0.27	0.25	0.49	0.26	0.15	0.28

Fertilisation variants: A_1=control; A_2 =NPK; A_3=N + CaCO3; A_4=A_2 + CaCO3; A_5= A_4 + manure
Cultivars: barley (jB1=Jagodinac, jB2=Grand); oats (oB3=Vranac) ; triticale (tB4=Knjaz)

The content of Cr in the tested samples of small grains (Tab. 1) indicates that Cr concentration in the aboveground vegetative biomass and kernel of small grains varied considerably depending on genotype and fertilisation treatment. The highest average Cr concentration in the vegetative biomass was recorded after NPK fertiliser treatment. Somewhat lower concentrations were found in the control and N+$CaCO_3$ treatment. NPK fertilisers, $CaCO_3$ and manure significantly reduced the absorption of this element in both the aboveground portions and the kernel of the tested cultivars. At a high redox potential and in the presence of organic matter, Cr(VI) is reduced to Cr(III). Cr(VI) is a soluble anion toxic to plants (Bartlett, 1991), and at an adequate pH and redox potential it is released into the soil solution in substantial amounts to be more intensely uptaken by plants. At a pH> 6, the dominant forms of Cr(VI) include: $HCrO_4^-$; $Cr_2O_7^{2-}$ and CrO_4^2. In contrast, Cr(III) ion has a lower solubility, and is available to plants only at biological pH values, if complexed to organic complexes of low molecular weight. Of the tested genotypes, the highest average Cr contents were found in the vegetative biomass of both barley cultivars (1.4 mg/kg in ‘Jagodinac’, range 0.5-2.2 mg/kg, and 1.1 mg/kg in ‘Grand’, range 1.0-1.3 mg/kg). In the other two genotypes of small grains (oat and triticale), the average contents and ranges of Cr were the same (0.5 mg/kg and 0.4-0.6 mg/kg, respectively), which is in agreement with the results obtained by other authors (Pelivanoska et al., 2011).

As reported in the literature, the average critical and toxic concentrations of Cr in crops are 1.0 mg/kg and 2.0 mg/kg dry matter, respectively (Pelivanoska et al., 2011), with the maximum tolerable level in feed of 3000 μg/g for chromium oxide and 1000 μg/g for chromium chloride (Vapa, 1997). The results and limit values for critical and toxic contents indicate that Cr content in the vegetative biomass of barley cv. ‘Jagodinac’ in the control and NPK fertiliser treatment was above toxic concentrations, due to which the vegetative biomass could not be used for safe food production. Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves, which may affect total dry matter production and yield. Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition (Shanker et al., 2005). Toxic amounts of Cr lead to plant stress due to the impeded absorption of almost all nutrients in the aboveground portions and K, P, Fe and Mg in the root (Kabata Pendias, 2011). Chatterjee and Chatterjee (2000) reported that an excess of Cr in plants caused poor protein formation resulting from the disruption of the N metabolism. Increased level of Cr^{6+} in nutrient solution decreased CO_2 assimilation and other

parameters associated with the photosystem of Lolium perenne leaves (Vernay et al., 2007). Conversely, the content of Cr in the other vegetative biomass samples was below critical levels, except in barley genotype ‘Grand’ where Cr levels were above critical and below toxic values. Previous literature data show that very low Cr levels of 1 to 2 mg/kg inhibits the growth of sensitive plant species (Kloke et al., 1984) and**can reduce dry biomass yield of plants by 10% (Jakšić et al., 2013).

Table 2. *Analysis of variance of the content Cr in small grain*

Effect of fertilisation on the content Cr				
Content	Mean sqr Effect	Mean sqr Error	F(df1,2) 3, 56	p-level
Cr in kernel	0.0174	0.022037	0.79110	0.503982
Cr in the above- ground portions	3.1490	0.130935	24.0498	0.000000
Effect of cultivars on the content Cr				
Content	Mean sqr Effect	Mean sqr Error	F(df1,2) 4, 55	p-level
Cr in kernel	0.18544	0.00990	18.72772	0.000000
Cr in the above- ground portions	0.53282	0.26633	2.00064	0.107193
Interaction of cultivar x year on the content Cr				
Content	Mean sqr Effect	Mean sqr Error	F(df1,2) 12, 40	p-level
Cr in kernel	0.038004	0.000907	41.91636	0.000000
Cr in the above- ground portions	0.399922	0.010050	39.79329	0.000000

* i ** *and* ** *= Significance for* F_{tab} *0,05 i 0,01*

The results of the present research show high differences in Cr accumulation between the aboveground portions and the kernel of small grains. Chromium is known to have poor availability for plant uptake, and its concentrations in plants are very low, ranging from 0.2 to 0.4 mg/kg (Pelivanoska et al., 2011). Chromium is slightly available to plants and not easily translocated within plants, thus it is concentrated mainly in roots, apparently because of the propensity of Cr^{3+} to bind to cell walls (Zayed et al., 1998). The amount of chromium in the aboveground portions of plants was higher than in the kernel, as follows: control - 3.70; NPK – 4.72; N+$CaCO_3$ – 1.79; NPK+$CaCO_3$ – 2.5; NPK+$CaCO_3$ +farmyard manure - 4.8. The highest effect on Cr mobility was produced by NPK fertilisers and manure. Under NPK+$CaCO_3$+manure treatment, its mobility was the lowest, since these fertilisers reduce chromium mobility (DTPA-extraction) (Singh et al., 2007). High application rates of organic, lime and phosphorus fertilisers are important in terms of environmental and health impacts as they mitigate the phytotoxic effects of Cr.

Conclusion

The Cr contents of the tested small grain samples suggest the following:

-Cr concentrations in the aboveground portions of the vegetative biomass and kernel of small grains varied considerably depending on genotype and fertilisation treatment.

-The results and limit values for critical and toxic contents indicate that Cr content in the vegetative biomass of barley cv. ‘Jagodinac’ in the control and NPK fertiliser treatment was above toxic concentrations, due to which the vegetative biomass could not be used for safe food production. In other samples, Cr content was below critical and toxic concentrations. Accordingly, the tested samples satisfied quality requirements with respect to Cr content.

-In terms of environmental and health impacts, the use of fertilisers, organic ones in particular, resulted in the reduction of Cr(VI) to Cr(III) and, hence, led to its decreased

solubility and toxicity, thus reducing its negative effect on plants and animal and human health.

-In all tested small grain genotypes, under all fertilisation treatments, Cr contents in the kernel were considerably lower than in the vegetative biomass, due to poor mobility and translocation, and were below critical and toxic levels. Therefore, the tested genotypes satisfied quality criteria with respect to Cr content.

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THE ROLE OF BIOFERTILIZATION IN IMPROVING APPLE PRODUCTION

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Abstract

Intensive agricultural production and increased synthetic nitrogen inputs affect environment through pollution with nitrogen metabolites. Over the past years, fruit growing practice has been focused on new concept which relies primarily on the application of microbial inoculants i.e. biofertilizers for gaining high yields and quality of fruits. In line with the concept, we conducted comparative study of the impact made by bio- (PGPR 1 and PGPR 2) and chemical fertilizers (Multi-Comp Base 14-13-20+2MgO+ME) on the yield-related characteristics of three apple cultivars (‘Gloster’, ‘Morens Jonagored’, ‘Hapke Delicious’). In addition, the paper presents the results related to the soil biological properties in the rhysosphere of the above-mentioned cultivars. The microbiological properties of soil were monitored by determining the total microbial count, numbers of soil fungi, actinomycetes, oligonitrophilic bacteria and azotobacter. The yield-related characteristics of the tested plants were evaluated for generative potential parameters i.e flowering intensity, fruit set intensity, final fruit set intensity and yield (kg tree^{-1}; t ha^{-1}).

The analysis of the results points to the fact that the introduction of biofertilization in apple production can meet the basic principles of sustainable agriculture, i.e. sustain stability and quality of yield, while at the same time preserving the ecological balance.

Key words: *biofertilization, apple, soil biological properties, generative potential*

Introduction

In recent years, intensive development of agricultural production has been directed towards optimisation of agro-technical measures in accordance with the modern trends of sustainable growth. Concerning these observations, Styła and Sawicka (2009) point out that improper implementation of agro-technical measures may cause disturbances in the functioning of the entire agro-system, with a negative impact on the development of soil microorganisms and fertility of farming land.

Nitrogen element (N_2) is the major component in the earth's atmosphere (approximately 80%). It is an essential element for most biochemical compounds in plants (proteins, nucleic acids, phytohormones, vitamins, etc.) which actively participate in biological processes responsible for growth and production. However, N_2 cannot be incorporated directly in plants' biological systems. In order to achieve this, it needs a reduction reaction – either chemically by adding hydrogen element to it or by recycling N_2 from the unavailable atmospheric form to available forms in the biosphere using the intermediary (Nitrogen fixing bacteria). In relation with this and to the purpose of securing a more efficient supply of fruits with necessary biogenic elements, apart from highly soluble chemical fertilisers, there is a recent trend of using microbial inoculants – biofertilizers. Abd El-Gleel Mosa et al. (2015) report that the biofertlization of fruits as a method of fertilization is considered a healthy alternative and/or supplement to mineral fertilizers. .

The active biological roles of N element on metabolism activities in fruit trees, with special emphasis on yield production, fruit quality and improving performance of roots, make N-fertilizers some of the most widely used fertilizers in the world (Mengel and Kirkby, 1987;

Westwood, 1993; Raese and Drake, 1997). In order to achieve and maintain the optimum balance between the growth and productivity in fruits, it is necessary to apply adequate combinations of pomo-technical measures and nutrition methods. Stiles and Reid (1991) state that a manifestation of deficiency symptoms or some of the toxicity symptoms related to excessive application of fertilizers, the plants are far from their optimum nutrition regime, either from the physiological or the economic and environmental point of view. In order to establish and maintain the optimum fertilisation regime of fruits, it is therefore necessary to monitor various parameters of growth in the plantations, from the early spring until the fruit-bearing season, while sustaining regular checks of the soil's productive capacity (Miller, 2002).

The generative potential of the apple is determined by numerous biotic and abiotic factors. According to Keserović et al. (2012), the main factors with an impact on bud formation are nutrition, foliage development, presence of fruits and seeds in fruits, as well as the growth of lateral shoots. On the other side, there are numerous references in the literature related to studies of the apple productivity, which point to the significant impact made by the genotype on this particular quality.

The aim of this paper is to examine the impact made by the fertilizer and apple genotype on microbiological activity of the soil and the most significant parameters of generative potential.

Material and method

The study included cultivars 'Gloster 69', 'Morens Jonagored', 'Hapke Delicious' and was conducted in the period 2011–2012, at the apple production plantation of the Fruit Research Institute in Čačak (west-central Serbia). The plantation was set up in 2006, using standard one-year-old plantings grafted on the M9 rootstock, planted at the 4 x 1 m planting distance (2.500 trees per ha^{-1}). The plantation was treated with standard pomo-technical and agro-technical measures, in accordance with the requirements of the apple as the fruit.

In the course of the study, two types of fertilizer were used – the chemical fertilizer (Multi-Comp Base 14-13-20 + 2MgO + ME) and two bio-fertilizers (PGPR 1 and PGPR 2).

The Multi-Comp Base 14-13-20 + 2MgO + ME is a highly soluble chemical fertilizer from the 'Haifa Chemicals Ltd' production programme. PGPR 1 represents a pure culture of *Gram*-negative nitrogen-fixing bacteria *Klebsiella planticola* TSHA-91. The bacteria titer in the inoculum was in the range 20-40 x 10^6 cm^{-3}. PGPR 2 is a microbiological fertilizer which contains bacteria of the *Azotobacter, Derxia, Pseudomonas* and *Bacillus* genera, as well as natural vitamins, enzymes and growth stimulators.

The fertilizers were applied using the drip irrigation system (in a concentration of 0.3% with 30 mm water (30 l /m^2)), applying the Venturi pipe, in three periods during the vegetation period (between the end of June until beginning of August). The irrigation periods were determined taking into consideration the condition of soil moisture, which was recorded using a tensiometer, as well as precipitation data.

Soil samples for microbiological analysis were taken at the end of the vegetation season. The microbiological analysis were performed in the Microbiology Laboratory of the Fruit Research Institute in Čačak, comprising determination of total numbers of microorganisms and numbers of fungi, using the method of dilution at corresponding solid culture media (Pochon and Tardieux, 1962). The total numbers of microorganisms were established on the media, for the total number of microorganisms. To determine the total numbers, 0.5 *mL* 10^{-6} of soil dilution was used. The number of soil fungi was determined using Chapec agar, whereas the number of actinomyces was determined on the synthetic Krasilnikov agar (1949); the number of oligonitrophilics and azotobacter was determined using the Fiodorov media. These groups of microorganisms were planted with 0.5 *mL* 10^{-5} of diluted soil suspension, except for azotobacter, where the planting was performed using the method of

fertile droplets with 0.2 *mL* suspension 10^{-2}. The incubation was performed over 7 days for the total numbers of microorganisms and actinomyces, over 5 days for fungi, 4–5 days for oligonitrophilics and 2 days for azotobacter, at the temperature of 28°C. The numbers off microorganisms have been calculated as per 1.0 *g* of absolutely dry soil.

Determining the intensity of flowering, as well as of the initial and final fruit set was accomplished using a 1-5 point scale. Yield per tree and unit of area was determined during the phase of physiological maturity by measuring the fruit mass and is expressed in kg tree^{-1}, i.e. t ha^{-1}.

The data was subjected to analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The Least Significance Difference (LSD) was used to compare treatment means and treatments declared different at $p = 0.05$ level of significance.

Results and discussion

Table 1 shows the results obtained in the study of the impact made by the applied fertilizer and genotype on the total numbers of microorganisms, fungi, actinomyces, azotobacter and oligonitrophilics in the soil of the experimental apple orchard.

Table 1. Impact made by the fertilizer and genotype on the overall number of microorganisms, number of fungi, actinomyces, azotobacter and oligonitrophilics in the soil of the experimental apple plantation

Factor		Total number of microorganisms	Number of			
			Fungi	Actynomicetes	Azotobakter	Oligonitrophils
		(CFU·10^6/g d.m. soil)	(CFU·10^5/g d.m. soil)	(CFU·10^5/g d.m. soil)	(CFU·10^2/g d.m. soil)	(CFU·10^5/g d.m. soil)
Fertilizer (A)	NPK 14:13:20	22.29±6.06 b	12.89±3.74 a	15.89±2.96 b	6.44±0.84 a	19.56±4.83 a
	PGPR 1	44.00±10.89 a	7.00±0.76 a	38.56±9.36 a	9.11±1.23 a	8.78±1.58 b
	PGPR 2	15.29±4.53 b	8.22±2.47 a	25.00±6.18 b	7.33±1.12 a	18.22±3.55 a
Cultivar (B)	‘Gloster 69’	14.20±2.90 b	8,56±1.49 ab	18.11±5.49 b	10.00±1.29 a	21.22±5.20 a
	‘Morens Jonagored’	24.22±8.66 ab	5.44±0,78 b	15.11±1.57 b	6.67±0,94 b	14.67±3.25 a
	‘Hapke Delicious’	41.11±9.49 a	14.11±3,67 a	46.22±7.79 a	6.22±0.55 b	10.67±1.54 a
ANOVA						
A		*	ns	*	ns	*
B		*	*	*	*	ns
A × B		ns	*	ns	ns	ns

The results of the variance analysis revealed a significant impact on the total number of microorganisms, number of actinomyces and oligonitrophilics, whereas the genotype had an important impact on the number of all of the analysed groups of microorganisms, with the exception of oligonitrophilics. A significant impact made by the interaction effect of the fertilizer/genotype was recorded only in the number of fungi in the soil.

Barabasz & Vořišek (2002) believe that rational chemical fertilization has a favourable effect on plant yielding. It must be stressed, however, that incorrect agro-technical treatments and irrational application of fertilization may cause disturbances in the functioning of the whole agrosystem and contribute to the development in soil environments of different noxious compounds (nitrozoamines, mycotoxins) acting unfavourably upon soil microorganisms, as well as upon cultivated plants and fertility of arable soils. Myśków and Kobus (1986), Wołoszyk and Nowak (1993), Myśków et al. (1996) found that repeated chemical

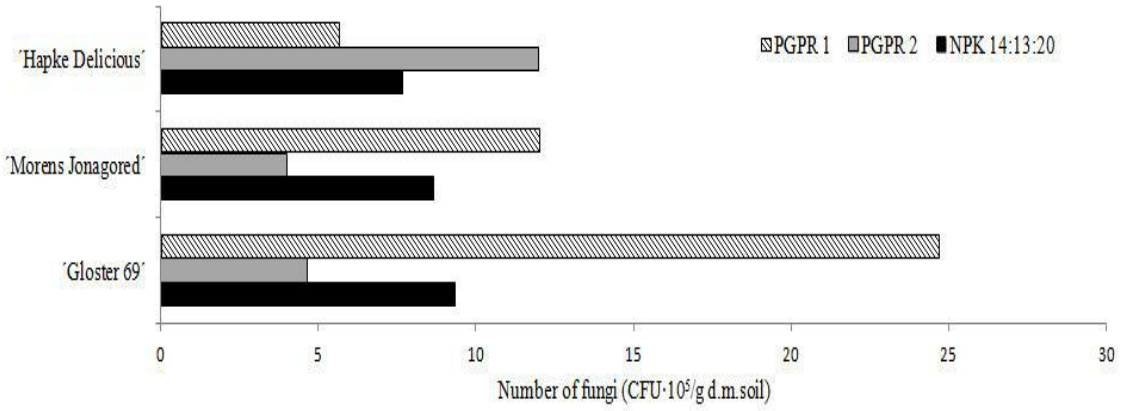
fertilization, particularly with high doses of nitrogen, can cause strong acidification of soils and increase the development of fungi which is in accordance with our results. Myśków et al. (1996) have found that the development of microfloral communities in the soil depends also on the type of the applied nitrogen fertilizer. These authors confirmed in their studies the presence of the greatest number of fungi in the soil fertilized with ammonium sulphate.

The values for the total number of microorganisms were in the range between 14.20 and 44.00. The largest total number of microorganisms was determined in the PGPR 1 treatment (44.00), which was considerably higher compared the chemical fertilizer treatment (22.29) and treatment with PGPR 2 (15.29). As for variances among the cultivars, significant differences in the total number of microorganisms in the rhizosphere were determined only between cv. ‘Hapke Delicious’ (41.11) and cv. ‘Gloster 69’ (14.20).

The number of fungi in the rhizosphere of the examined apple cultivars ranged between 5.44 and 12.89. Significant differences in the number of fungi in the soil existed between the ’Hapke Delicious’ and ‘Morens Jonagored’ cultivars, which can be explained by the differences in the exudates production. Comparing the interaction effects of the applied fertilisers and the genotypes involved, a significantly larger number of fungi was recorded in the PGPR 1 treatment in cv. ‘Gloster 69’ (Graph 1).

A significantly higher number of actinomyces in the apple rhizosphere was recorded in the PGPR 1 treatment (38.56), whereas the best results from the aspect of genotype effect were demonstrated by cv. ‘Hapke Delicious’ (46.22). The number of azotobacter in cv. ‘Gloster 69’ (10.00) was significantly higher, compared to cvs. ‘Morens Jonagored’ and ‘Hapke Delicious’ (6.67 and 6.22, respectively).

The most favourable impact on the number of oligonitrophilics in the apple rhizosphere as made by the chemical fertilizer and PGPR 2 (19.56 and 18.22, respectively), whereas the lowest number of oligonitrophilics was recorded in the treatment with PGPR 1 (8.78).



Graph 1. Impact made by interaction effect fertilizer/genotype on the number of fungi in the soil of the experimental apple plantation

Based on the results obtained in studying the parameters of the apple generative potential (Table 2), it can be inferred that the fertilizer, genotype and their interaction effect made no significant impact either on the intensity of flowering or the initial and final fruit set.

Table 2. Impact made by the fertilizer and genotype on the generative potential parameters in apple

Factor		Flowering intensity (1–5)	Fruit set intensity (1–5)	Final fruit set intensity (1–5)	Yield (kg tree^{-1})	Yield (t ha^{-1})
Fertilizer (A)	NPK 14:13:20	3.56±0,33 a	2.57±0.32 a	2.10±0.29 a	7.99±1.64 a	20.04±4.09 a
	PGPR 2	2.97±0.28 a	2.13±0.24 a	1.97±0.17 a	8.15±1.35 a	20.38±3.37 a
	PGPR 1	3.63±0.26 a	2.40±0.33 a	2.33±0.18 a	8.32±1.10 a	20.79±2.76 a
Cultivar (B)	‘Gloster 69’	3.20±0.26 a	2.57±0.17 a	2.20±0.09 a	11.66±1.37 a	29.21±3.39 a
	‘Morens Jonagored’	3.13±0.37 a	2.40±0.36 a	2.17±0,30 a	6.82±1.04 b	17.04±2.60 a
	‘Hapke Delicious’	3.83±0.22 a	2.13±0.33 a	2.03±0.23 a	5.98±0.65 c	14.96±1.63 b
ANOVA						
A		ns	ns	ns	ns	ns
B		ns	ns	ns	*	*
A × B		ns	ns	ns	ns	ns

The variance analysis revealed that significant differences in the measured yield per tree and yield per ha existed only between the examined cultivars. A significantly higher yield per tree was determined in cv. ‘Gloster 69’ (11.66 kg tree^{-1}), compared to the two other cultivars under consideration. In addition to this, the highest yield per ha was determined in cv. ‘Gloster 69’ (29.21 t ha^{-1}), which was not considerably different compared to the yield of cv. ‘Morens Jonagored’. The lowest yield per unit of area was recorded in cv. ‘Hapke Delicious’ (14.96 t ha^{-1}). Similar conclusions were made by Aslantaş et al. (2007) in their study of the rootstock (M9, MM106), of the cultivars under consideration (‘Granny Smith’ and ‘Stark Spur Golden’) and the impact made by PGPR (OSU-142, OSU-7, BA-8 i M-3) on apple growth and yield. Favourable effects of sensible application of fertilizers on the volume of yield have been further confirmed by the results obtained by Barabasz and Vořišek (2002).

Conclusion

Based on the results obtained in the research, it is possible to reach the following conclusions:
The most favourable impact on biological activity of the soil in the apple plantation was achieved in the treatment deploying PGPR1;
The most favourable effect of the genotype on the biological activity of the soil was determined in cv. ‘Hapke Delicious’;
The most favourable interaction effect was determined in the PGPR 1/‘Gloster 69’ combination.
The largest yield per tree and unit of area was obtained in cv. ‘Gloster 69’.

The analysis of the results points to the fact that the introduction of biofertilization in apple production can meet the basic principles of sustainable agriculture, i.e. sustain stability and quality of yield, while at the same time preserving the ecological balance.

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VARIATION OF MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF MAIZE HYBRID SEED OVER GROWING LOCATIONS

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Abstract

Physiological and morphological traits of seed and their variability in dependence on the growing location were studied in 2014. The analyses were carried out on hybrid seed of three commercial hybrid combinations produced in three locations. The following seed traits were observed under laboratory conditions: germination energy, total germination and 1000-seed weight. The highest, i.e. lowest values of germination and germination energy were recorded in the hybrid H3, i.e. H1, respectively. The greatest 1000-seed weight was detected in the hybrid H3. With regard to the growing location, the highest values of germination and germination energy were recorded in the location B (97.166%), while the location A was most suitable for the expression of 1000-seed weight. The greatest 1000-seed weight amounted to 394.67 g. The hybrid combination × location interaction significantly affected all three traits. The highest energy and germination were recorded in the hybrid H1 in the location B. The hybrid combination H2 and the location C had the highest effect on 1000-seed weight. This trait mostly varied in the hybrid combination H3 in the location C. Average values of germination energy, total germination and 1000-seed weight very significantly depended on the hybrid combination and the growing location.

Key words: *hybrid, location, seed trait*

Introduction

Hybrid maize seed is produced under diverse agro-ecological and agro-technological conditions and therefore traits of such seed depend on many factors: genotypic combinations of parental inbreds, coincidence of flowering times of parental inbreds, duration of pollination in seed crops, duration of seed filling, duration of maturation of formed and filled seed, moisture content in physiologically mature seed at harvest, seed maturation, seed infection by disease-causing agents, presence of pests. It was determined that numerous factors affect the process of maize seed germination both, individually and interactionally, while obtained results refer to seed as an entity (*Antonić et al., 2003, Dačić et al., 1997, Sabovljević et al., 1997.*). Agro-ecological conditions depend not only on meteorological and edaphic conditions in a region but also on applied cropping practices in the production of seed crops. Furthermore, all traits of hybrid maize seed depend on a genotypic combination and interactions of this combination with agro-ecological conditions during the growing season of seed crops. *Lee et al. (2002)* presented important differences in cold tolerance related to a genotype. These differences manifested in different rates of the root development but also in development rates of the above-ground shoots. Based on the stated, traits of produced hybrid maize seed depend on procedures absolutely controlled by humans, on ecological conditions influenced, to a certain extent, by humans, on traits of parental inbreds and on interactions among all stated factors.

The aim of the present study was to observe effects of growing locations on physiological and morphological traits of maize seed: germination energy, total germination and 1000-seed

weight, as well as, their variatons in relation to the production region in the year of investigation.

Material and Methods

The study was carried out with three commercial single-cross combinations of maize of different FAO maturity group (FAO 300, FAO 400 and FAO 600) derived at the Maize Research Institute, Zemun Polje.

Hybrid seed of the F1 generation for each hybrid combination was produced in three locations, during 2014: SC341(H1) - Sanad (A), Žarkovac (B) and Feketić (C), SC427 (H2) - Sanad (A), Žarkovac (B) and Sivac (C), SC666 (H3) - Sanad (A), Žarkovac (B) and Feketić (C). In the production of seed crops, all standard and prescribed cropping practices and procedures were applied in due time and in the right way in each of locations. Harvest of seed ears was done by maize pickers. Husking was performed immediately prior to artificial drying. After drying, ears were shelled and the natural seed material was cleaned. Submitted samples of 25-30 kg were drawn out of natural seed material for each hybrid combination and each production location. Non-destructive methods were applied to study hybrid maize seed. Seeds were observed and evaluated as entities. Total germination, germination energy and 1000-seed weight were determined under laboratory conditions according to the Regulation on Seed Testing Quality of Agricultural Crops 47/87 and ISTA Rules (currently valid edition). The standard method for total germination and germination energy was applied (t_1=20/30^{o}C and (16:8) light : dark photoperiod). The first, i.e. final count were done on the forth, i.e. seventh day, respectively.

Each of obtained parameters was statistically processed by descriptive statistics for parameters at the annual level. Differences among analysed maize hybrids as well as their interactions were evaluated by the analysis of variance. Gained experimental data were processed by the appropriate mathematical and statistical methods using the statistic package STATISTICA 10.0 for Windows.

Results and Discussions

Table 1 illustrates data of means of qualitative and quantitative seed traits. In all three hybrids and in each of three locations standards prescribed by the Regulation on Seed Testing Quality of Agricultural Crops were met, except for H1 in the location A (germination energy was bellow 90%). Results obtained in the fields and the laboratory confirmed better germination and germination energy at higher temperatures (*Sabovljević et al.,* 1997; *Rosić*, 1959; *Popović*, 1982; *Pinnel*, 1949). According to Živanović et al. (2012), Marić et al. (2013) daily temperatures in the stage of germination and emergence should be 8-10^{o}C for germination and 10-12^{o}C for emergence in order to have successful maize production. The expression of germination energy and total germination in this study was uniform, with variations less than 2.1%, while the third observed trait was characterised by a significantly greater variation in relation to both factors. The analysis of the first factor (hybrid combination) shows that the hybrid H3 had the highest mean for germination energy and germination of 96.9%, while 1000-seed weight amounted to 432.6 g in the hybrid H2. Furthermore, the analysis of different groups of hybrids points out to the effect of a hybrid combination on germination and germination energy (*Tabaković et al., 2015*). The best conditions for germination energy and total seed germination were registered in the location B with the mean of these traits amounting to 97.2%. The highest mean value of 1000-seed weight (394.6 g) was estimated in the location A. *Shien* and *Mc Donald* (1982) determined the influence of seed size, shape and treatment on seed quality of two inbred lines.

Table 1. Means and variations of seed traits ($S\overline{x}, \overline{X}$)

	H1			H2			H3			$\overline{X}$,$S\overline{x}$		
	ge	g	sw	ge	g	sw	ge	g	sw	ge	g	sw
A	85.0 ±2.4	87.1 ±1.6	364.2 ±13.5	94.1 ±0.4	94.1 ±0.4	443.7 ±49.2	97.1 ±0.4	97.1 ±0.4	376.0 ± 10.1	92.0 ±2.1	92.7 ±1.6	394.6 ±17.1
B	98.4 ±0.3	98.4 ±0.3	322.2 ±12.8	96.8 ±0.5	96.8 ±0.5	403.2 ±43.1	96.3 ±0.8	96.3 ±0.8	350.7 ± 12.9	97.2 ±0.6	97.2 ±0.6	358.7 ±16.6
C	97.7 ±0.4	97.7 ±0.4	308.7 ±14.2	96.2 ±0.5	96.2 ±0.5	451.0 ±36.4	97.5 ±0.4	97.5 ±0.4	373.4 ± 10.2	97.1 ±0.4	97.1 ±0.4	377.7 ±22.0
	93.4 ±2.4	94.4 ±1.9	331.7 ±15.1	95.7 ±0.6	95.7 ±0.6	432.6 ±14.8	96.9 ±0.5	96.9 ±0.5	366.7 ±11.4	$\overline{X}$.$S\overline{x}$		

A, B, C - location, H1, H2, H3 - hybrid combination, ge - germination energy, g - total germination, sw - 1000-seed weight, $\overline{X}$-mean, $S\overline{x}$-standard error

The effect of hybrid combinations and locations on studied physiological and morphological traits was determined by the two-factorial analysis of variance (Table 2). The statistically significant difference among observed groups of hybrids and locations was estimated at the $p<0.05$. Moreover, the influence of interactions of factors on the expression of seed traits was also determined ($F=23.25$, $p<0.05$, $F=19.12$, $p<0.05$). An effect size measure was done by partial eta-squared. The eta-squared values ($\eta=0.535$, $\eta=0..486$) point to great effects of hybrid combinations and locations on trait variances. The highest partial effect was estimated for the hybrid combination on 1000-seed weight ($\eta=0.542$).

Table 2. Two-factorial analysis of variance and partial eta-squared

Factor	Seed trait	d.f.	Mean squares	F-test	Partial eta-squared
hybrids	germination	2	49.411	9.882*	0.196
	energy	2	81.378	9.309*	0.187
	1000-seed weight	2	78756.908	47.852*	0.542
location	germination	2	192.144	38.429*	0.487
	energy	2	258.411	29.560*	0.422
	1000-seed weight	2	9718.047	5.905*	0.127
hybrids * location	germination	4	116.294	23.259*	0.535
	energy	4	167.161	19.122*	0.486
	1000-seed weight	4	3630.965	2.206	0.098

Additional comparisons using the $LSD_{0,05}$ test (Table 3) indicated that means for all three traits differed significantly over hybrids, except in germination energy between the second and the third hybrid. The significant differences in germination energy were also detected between the locations A and B and A and C. On the other hand, significant differences in 1000-seed weight were recorded only between locations A and C.

Table.3 Significance of differences at the 0.05% probability level

Seed trait		(I) hybrid	(J) hybrid	Difference between means (I-J)	95% confidence interval		(I) location	(J) location	Difference between means (I-J)	95% confidence interval	
					Lower limit	Upper limit				Lower limit	Upper limit
g	LSD	1	2	-1.30*	-2.44	-0.15	1	2	-4.40*	-5.54	-3.25
			3	-2.56*	-3.71	-1.41		3	-4.36*	-5.51	-3.21
		2	1	1.30*	0.15	2.44	2	1	4.40*	3.25	5.54
			3	-1.26*	-2.41	-0.11		3	0.03	-1.11	1.18
		3	1	2.56*	1.41	3.71	3	1	4.36*	3.21	5.51
			2	1.26*	0.11	2.41		2	-0.03	-1.18	1.11
ge	LSD	1	2	-2.00*	-3.51	-0.48	1	2	-5.10*	-6.61	-3.58
			3	-3.26*	-4.78	-1.74		3	-5.06*	-6.58	-3.54
		2	1	2.00*	0.48	3.51	2	1	5.10*	3.58	6.61
			3	-1.26	-2.78	0.25		3	0.03	-1.48	1.55
		3	1	3.26*	1.74	4.78	3	1	5.06*	3.54	6.58
			2	1.26	-0.25	2.78		2	-0.03	-1.55	1.48
sw	LSD	1	2	-100.90*	-121.74	-80.06	1	2	35.97*	15.13	56.81
			3	-34.97*	-55.81	-14.13		3	16.95	-3.88	37.79
		2	1	100.90*	80.06	121.74	2	1	-35.97*		-15.13
			3	65.93*	45.08	86.77		3	-19.02	-39.86	1.82
		3	1	34.97*	14.13	55.81	3	1	-16.95	-37.79	3.88
			2	-65.93*	-86.77	-45.08		2	19.02	-1.82	39.86

g-total germination, ge- germination energy, sw- 1000-seed weight

Conclusion

Studies on physiological and morphological seed traits showed that these traits are commercially important. An appropriate number and arrangement of plants in mercantile crops of maize hybrids are primarily provided by sowing seeds of good physiological, physical and mechanical traits. Our studies pointed out that variations in seed traits depended not only on genetically predetermined limits, but also on different production conditions. Effects of hybrid combinations and locations were great on all traits. According to obtained results, germination energy and total germination were more dependent on production conditions, i.e. locations (η=0,487 and η=0,422, respectively), while 1000-seed weight depended more on genetic performances of a hybrid combination (η=0,542). The significance of a hybrid combination and a location was confirmed by the analysis of variance, while the significance of obtained differences was confirmed by the LSD test.

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SOME MORPHOLOGICAL AND PRODUCTIVE TRAITS OF WINTER RYE DEPENDING ON TILLAGE DEPTH

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Abstract

Besides it is a significant cereal for bread making, rye is characterized by favorable nutritive properties. Furthermore, it also can be used as feed, then for production of alcohol, starch and vinegar, cellulose, lignin, while rye germ is used in pharmaceutical industry. This study has been aimed to investigate the effect of soil cultivation depth on some morphological and productive traits of winter rye in hilly and mountain areas of north Kosovo. The trial was carried out during 2009/10 and 2010/11 in mountain area of north Kosovo, at 630 m of altitude. It was set in random complete block design with three replications. Cultivar of winter rye Raša was used for the trial. Three depths of soil cultivation were investigated (disc harrowing to 10-15 cm of depth, plowing to depth of 20 cm and plowing to depth of 30 cm). Dependence on tillage depth of the following parameters was observed: plant height, spike length, number of grains per spike, absolute mass of grain, hectoliter mass of grain and grain yield at 14% of moisture. The data were collected for both years and statistically processed by analysis of variance. Results of the study showed that soil cultivation depth had significant effect on the all investigated parameters. Significant differences were observed in plant height, spike length and number of grains per spike between the variant where tillage was done only by disc harrowing and the variant with plowing to 20 cm of depth. Absolute and hectoliter mass of grain were the highest in tillage to 30 cm of depth, with significant differences only in regard to disc harrowing, while between disc harrowing and plowing to depth of 20 cm there was not any statistically significant difference. Grain yield was the highest in tillage to 30 cm of depth (2450 kg ha^{-1}) and was significantly higher in regard to tillage only by disc harrowing (1840 kg ha^{-1}). There was no statistically significant difference in grain yield between plowing to 20 and 30 cm of depth. In order to get rye grain yield of a satisfactory level, one should not do soil cultivation at depth lower than 20 cm.

Key words: *rye, number of grains per spike, absolute mass, hectoliter mass, grain yield*

Introduction

Rye (*Secale cereale L.*) is an annual plant from the family *Poaceae*. It is important as bread making cereal crop, especially in northern parts of Russia and northern Europe (Poland, Germany, Sweden, etc). Bread made of rye is tasteful, nutritious, and can keep freshness for a long time. It contains lower amount of starch than wheat, so bread made of rye is recommended in diet for diabetics. It is also an excellent forage crop, regardless as green forage, or as bran, flour and grain. Straw is good for house roofs, and for making straw hats or mattings. Rye also can be used for production of alcohol, starch and vinegar, cellulose, lignin, while rye germ is used in pharmaceutical industry. Rye is very rich by vitamins A, B and E, and has a great importance, so as human and animal food, as in industry (Oelke, 1990).

Among the other cereal crops, the total area sown by rye in the world is at the sixth place, just behind wheat, maize, rice, barley and oats (Todorović and Komljenović, 2009).

In our country rye is more rarely grown than the other small grain crops, but recently there is a greater interest for its growing. According to Jevtić (1992), the average rye grain yield in

countries of former Yugoslavia is quite low, which means that rye production did not earn enough attention, so in choosing genotypes as concerning agrotechnique.
The data reported by Biberdžić et al. (2011) point that rye has good competitive properties and the lowest degree of weed infestation comparing with the other small grain crops.
This study has been aimed to investigate the effect of soil cultivation depth on some morphological and productive traits of winter rye in hilly and mountain areas of north Kosovo.

Material and methods

The trial was carried out during 2009/10 and 2010/11 in mountain area of north Kosovo, at 630 m of altitude. It was set in random complete block design with three replications. Cultivar of winter rye Raša was used for the trial. Previous crops were fertilized by 20 t ha^{-1} of manure each, while mineral fertilizers and chemical preparations for plant protection were not applied. Sowing was carried out during the last decade of October, and previous crops were potato and maize. Three depths of soil cultivation were investigated (disc harrowing to 10-15 cm of depth, plowing to depth of 20 cm and plowing to depth of 30 cm). Dependence on tillage depth of the following parameters was observed: plant height, spike length, number of grains per spike, absolute mass of grain, hectoliter mass of grain and grain yield at 14% of moisture. The data were collected for both years and statistically processed by analysis of variance, while statistical significance was estimated by LSD test.

Soil conditions

Table 1.Chemical properties of the soil

Depth (cm)	pH		Humus	N	P_2O_5	K_2O
	H_2O	KCl	%	%	mg/100 g	mg/100 g
0-20	6.23	4.80	4.66	0.233	11.33	27.84

The data presented in table 1 point to the soil as an acid one. It is well-supplied by humus and nitrogen. Good humus supply is caused by application of manure in the previous years. Phosphorus content is low, at the line between poor and moderate supplied soils, while this soil is well-supplied by potassium. Although this soil has been acid, Nožinić et al. (2009) reported that rye genotypes have been tolerant to extremely acid soil environment, giving satisfactory grain yield in extremely droughty years.

Results and discussions

Rye grain yield, as well as one of the other grain crops, depends on many factors, primarily on cultivar, agroclimatic conditions of the area observed, agrotechnique, etc. One of important production factors is agrotechnique, especially tillage and soil preparation. Often, for many reasons (previous crop, shortage of time and machinery), we are not able to do basic soil cultivation on time. Therefore, not so rare, basic soil cultivation is avoided or minimized, which can cause a low and unstable grain yield. Table 2 gives the influence of tillage depth on some morphological traits of winter rye.

Table 2. Morphological traits of the rye, depending of tillage depth

Tillage depth (variants)	Morphological traits of the rye	
	Plant height (cm)	Spike length (cm)
Disc harrowing to depth of 10-15 cm	131.2	10.5
Plowing to depth of 20 cm	138.0	11.8

Plowing to depth of 30 cm	141.4	12.4
Average	136.8	11.5
LSD 0.05	4.45	1.25
LSD 0.01	6.74	1.89

Soil cultivation depth had significant effect so on plant height, as on spike length and number of grains per spike. The average rye plant height for the all cultivation depth values was 136.8 cm. The highest one was observed in soil cultivation variant III, amounting 141.4 cm, and the lowest one in the tillage variant I, where it was 131.2 cm. Rye plants from cultivation variants II and III had high-significantly greater plant height in regard to the plants of tillage variant I, while between variants II and III there was not any significant difference.

Plant height is an important trait, because plants with lower stem are more resistant to lodging. Milovanović et al. (2005) stated that this cultivar is 140 cm high in average, and shows a good resistance to lodging.

The average spike length amounted 11.5 cm. The lowest spike length (10.5 cm) had plants from tillage variant I, and it was significantly lower in regard to the plants from variant II, and high-significantly lower in regard to the plants from tillage variant III. There was not any significant difference in spike length between plants from tillage variants II and III.

Table 3 gives number of grains per spike, absolute and hectoliter mass of rye grain, that are important physical grain properties, as well as rye grain yield, desired by the all rye producers. Tillage depth showed significant effect on all the mentioned parameters.

Number of grains per spike is a very important and, besides number of plants per square unit and absolute mass of grain, one of the three crucial factors for total grain yield amount. The highest number of grains per spike (52.5) had plants from soil cultivation variant III, and the lowest one (46.3) plants from tillage variant I. There was not any significant difference between tillage variants II and III.

Absolute mass of grain is one of the three crucial factors influencing total amount of rye grain yield. It ranged from 39.3 g in tillage variant I to 41.8 g in tillage variant III. Absolute mass of grain achieved in soil cultivation variant III was high-significantly greater in regard to the cultivation variant I. There was not any significant difference in absolute mass of grain between tillage variants I and II, as well as between variants II and III. Hectoliter mass, as a parameter of grain largeness and fulfillment, showed similar tendency as absolute mass of grain.

Table 3. Important productive traits of the rye, depending of tillage depth

Tillage depth (variants)	Productive traits of the rye			
	No of grains per spike	1000 grain mass (g)	Hectoliter mass (kg)	Grain yield (kg ha^{-1})
Disc harrowing to depth of 10-15 cm	46.3	39.3	72.6	1840
Plowing to depth of 20 cm	50.8	41.2	74.6	2330
Plowing to depth of 30 cm	52.5	41,8	75.9	2450
Average	49.8	40.7	74.3	2330
LSD 0.05	4.32	2.05	3.00	380.10
LSD 0.01	6.55	3.11	4.55	575.90

Grain yield is the trait desired by the all rye producers. Soil cultivation depth had highly significant effect on rye grain yield. So that, the highest rye grain yield (2450 kg ha-^{1}) was achieved at the cultivation variant III, and the lowest one (1840 kg ha^{-1}) at the cultivation variant I, so the difference in grain yield between tillage variants I and III was 610 kg ha^{-1}. Difference in grain yield between tillage variants I and II was statistically significant, while the difference between tillage variants I and III was statistically highly-significant. There was not any significant difference between tillage variants II and III. The reported results point to importance of soil cultivation depth in formation of rye grain yield. It is often case that rye, as the other small grain crops, is sown without basic soil cultivation, i.e. called in slang, "under the disc harrow". It is usually done when farmers are in shortage of time (late harvest of previous crop), which is often case in our conditions. Result of such procedure is much lower grain yield, which was proven by our study. Our study shows that soil cultivatition ought to be done, at least, down to 20 cm, in order to achieve satisfactory results. Previous reports state that basic soil cultivation should be done at least 15 days before sowing, at the depth of 30 cm. Reasons for lower rye grain yield observed in our investigation could be found in a later sowing term and the absence of mineral fertilizers. We must point that before previous crops 20 t ha-^{1} of manure was applied, and chemical preparations for plant protection were not applied, so this production could be considered as the organic one.

Having in mind the cultivar Raša, Milovanović et al . (2005) recommend it to be sown during the end of September and the beginning of October.

Biberdžić et al. (2011) reported that, by application of mineral fertilizers with increased dose of phosphorus, they reach rye grain yield of 2913 kg ha^{-1}. Rye grain yield observed in our study was slightly lower than the average rye grain yield in Serbia during 2009, which was 2500 kg ha^{-1} (Statistical Yearbook of Serbia, 2010). Oljača et al. (2011) stated that the best rye grain yield can be reached by combined application of organic biostimulators and soil improving agents.

Conclusions

On the basis of the study we can conclude the following:

Tillage depth showed a significant effect on the all studied parameters;

Plant height, spike length and number of grains per spike at variants II and III of soil cultivation had significantly higher values in regard to those from variant I of soil cultivation;

Absolute and hectoliter mass observed in tillage variant III was significantly higher than in tillage variant I, while between tillage variants I and II, as well as between variants II and III, there was not any statistically significant difference;

Difference in grain yield between soil cultivation variants I and II was statistically significant, while difference between variants I and III was highly significant.

Difference between soil cultivation variants II and III was not statistically significant;

The highest values of the all studied parameters were observed at the variant III of soil cultivation;

Difference between soil cultivation variants I and III amounted 610 kg ha^{-1};

The observed grain yield in tillage variant II can be regarded as satisfactory one, having in mind that no mineral fertilizers and chemical preparations were used;

In order to keep rye grain yield at a desired level, one ought to do soil cultivation at the depth between 20 and 30 cm, together with sowing during optimal terms.

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POMOLOGICAL PROPERTIES AND YIELD OF INTRODUCED APPLE CULTIVARS IN THE REGION OF WESTERN SERBIA

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Abstract

The paper presents the results of a three-year study into pomological properties and yield of two intense-colouring sports of ‘Gala’ and ‘Elstar’ (‘Gala Must’ and ‘Red Elstar’, respectively) and two scab resistant (‘Rajka’ and ‘Topaz’) apple cultivars which were grown under agro-environmental conditions of Čačak, Western Serbia. Regarding harvest maturity, Gala Must’ and ‘Red Elstar’ belong to the group of autumn cultivars (18th August and 2nd September, respectively), while ‘Rajka’ and ‘Topaz’ are winter cultivars (18th and 21st September, respectively). Average fruit weight ranged from 143.33 g (‘Rajka’) to 157.56 g (‘Gala Must’), height from 54.21 mm (‘Topaz’) to 61.33 mm (‘Gala Must’) and width from 67.77 mm (‘Rajka’) to 71.80 mm (‘Red Elstar’). The best fruit quality among the assessed cultivars, measured by the chemical composition, was found in the ‘Topaz’ (soluble solids content – 14.86%; total sugars and acids content – 12.50% and 0.54%, respectively). ‘Gala Must’, ‘Topaz’ and ‘Rajka’ showed high cropping potential, although ‘Gala Must’ had the highest yield (24.63 kg tree^{-1} and 61.58 t ha^{-1}). Based on overall results, the assessed cultivars may contribute to the advancement of the Serbian apple assortment, while ‘Gala Must’ and ‘Topaz’ can be recommended for commercial production.

Keywords: *Malus* × *domestica*, cultivar, sport, pomological properties, yield

Introduction

Apple (*Malus* × *domestica* Borkh.) is economically the most important species of genus *Malus* Miller in the Republic of Serbia, with the total cultivated area reaching around 43,250 ha and the average production of 259,671 tons (for the period 2009–2013; Faostat, 2015). Lukić (2006) and Milatović *et al*. (2009) reported that ‘Idared’ is still the most popular cultivar, representing around 50% in the apple assortment structure of our country. Over the last few years more intensive orchards with introduced cultivars have been established and new technologies have been applied (Nikolić *et al*., 2012).

The modern apple production demands that a cultivar have consistent yields and quality fruit, be suitable for manipulation, storage and shipping. In addition to this, from the aspect of rational use of pesticides and environmental protection, growing cultivars resistant to pests and causal agents of the most serious diseases is one of the recent trends in apple production. Brown *et al*. (1999) pointed out that in determining the commercial potential of any new apple cultivars, three questions should be asked: what quality can you obtain with this cultivar; does that quality set it apart; is that qualityconsistent. Estimates of cultivar trends for 34 major producing countries (excluding China) were published by O′Rourke (2001), who reported that the volume of production of ‘Red Delicious’, ‘Golden Delicious’ and ‘Granny Smith’ will be stable in the next few years and production of ‘Gala’, ‘Braeburn’ and ‘Pink Lady®’ will record significant gains in volume.

‘Gala’ and ‘Elstar’ belong to the group of 12 apple cultivars that are currently the most important in the world trade (Hampson and Kemp, 2003). Both cultivars are prone to producing red colour sports, whose red colour – despite the fact that it does not affect the eating quality – influences consumer decisions to buy apples and thus indirectly determines

the profits of growers (Iglesias and Alegre, 2006). Recently, there has been a growing popularity of winter cultivars of Czech origin, such as ‘Rubinola’, ‘Rajka’, ‘Goldstar’ and ‘Topaz’ (Sosna, 2014). In addition to their other features, these cultivars can be compared even with ‘Jonagold’ in terms of taste, appearance and storage properties of fruits.
Paper aims at studing the pomological (morphometric and chemical) properties and yield of two intense-colouring sports of ‘Gala’ and ‘Elstar’ (‘Gala Must’ and ‘Red Elstar’, respectively) and two scab resistant (‘Rajka’ and ‘Topaz’) cultivars in the region of Čačak (Western Serbia). Based on these results, it will be established whether agro-ecological conditions of Western Serbia are suitable for their growing, while making it possible from the practical point of view to recommend the best cultivars for commercial growing.

Materials and methods

Plant material

The study was conducted over the three-year period at the apple trial at the Preljinsko brdo facility (43°53' N; 20°21' E; 350 m above the sea) of the Fruit Research Institute, Čačak, Republic of Serbia. The trial was set up using the ‘knip’ seedlings, grafted on the M9 rootstock, at the 4 × 1 m planting distance. Slender spindle was the applied training system.
Two of the intense-colouring mutants of ‘Gala’ (‘Gala Must’) and ‘Elstar’ (‘Red Elstar’) cultivars were examined: ‘Regal Prince’ (‘Gala Must’®) is the dark-red mutant of ‘Gala’ (‘Kidd’s Orange Red’ × ‘Golden Delicious’), featuring firm, sweet and juicy fruits, discovered by Pepinieres Davodeau, Angers, France (Rutkovski *et al*., 2005); and ‘Red Elstar’ is a mutant of ‘Elstar’ (‘Ingrid Marie’ × ‘Golden Delicious’), discovered in Holland (Zeeland) in 1981, differing from the standard cultivar in its intense red colour, covering almost the entire surface of the fruit, while at the same time having the same cropping potential as ‘Elstar’ (Goddrie, 1994).
Two scab resistant cultivars developed at Institute of Experimental Botany, Experiment Station Střížovice, Czech Republic were also assessed: ‘Rajka’ (‘Champion’ × UEB 1200/1) is an early-winter, dessert cultivar with outstanding colour, good, rather sweet flavour and medium storage life (Lukić and Marić, 2012); and ‘Topaz’ (‘Rubín’ × ‘Vanda’) is a late, long-keeping apple cultivar with an excellent eating quality (Lukić and Marić, 2012).

Methods

Within the three-year period, the pomological (morphometric and chemical) properties and yield of the aforementioned apple cultivars were analysed.
Morphometric characteristics of the fruits were determined using the standard methods on a sample consisting of 75 fruits, by monitoring the following parameters: fruit weight, height and width, fruit shape index, length of stalk and number of seeds per fruit.
The following parameters were assessed in order to determine the fruit chemical composition: content of soluble solids (using the ‘Carl Zeiss’ binocular refractometer); content of total and invert sugars (according to the Luff-Schoorl method); sucrose content (calculated as the difference between the total and invert sugars, multiplied by coefficient of 0.95); content of total acids, expressed in malic acid (titration of 0.1 N NaOH with the presence of phenolphthalein as indicator); pH value of the fruit juice (CyberScan 510 pH meter).
The yield of the examined cultivars (kg tree^{-1} and t ha^{-1}) was determined by measuring the yield of individual trees of each cultivar, together with calculating the yield per unit of area.

Statistical analysis of data

The statistical significance of the quantitative values was determined using the Fisher model of variance analysis (ANOVA) of two-way factorial experiment, applying the F test for $P \leq 0,05$ and $P \leq 0,01$. In the cases when the F test revealed significance, differences of arithmetical means and their interaction effect were further tested using the test of least significant differences (LSD test)

for significance threshold of $P \leq 0,05$ and $P \leq 0,01$. SPSS statistical software package (SPSS. Inc., Chicago, IL) was used for the data analysis.

Results and discussion

Pomological properties of the introduced apple cultivars

The examined apple cultivars demonstrated a regular order of fruit ripening times over the three years of study, while it was also observed that during the second and the third years off study, the fruit maturity occurred later in the ripening season (Table 1). On the average, the earliest harvest maturity was recorded in the fruits of 'Gala Must' (18th August). This cultivar belongs to the autumn cultivars, together with 'Red Elstar' (2nd September). The latest ripening time was recorded in 'Topaz' (21st September) which, together with 'Rajka' (18th September), belongs to winter apple cultivars. Fruits of 'Gala Must' reached harvest maturity in the period between 14th and 23rd August, which is in agreement with the data reported by Milatović *et al*. (2009) for this cultivar in the central Šumadija region. The average ripening time for 'Topaz' grown in the Čačak agro-ecological conditions is earlier compared to the ripening time for the conditions in Slovenia, where according to Beber (2009) this cultivar reaches harvest maturity nine days later compared to 'Golden Delicious'. Godec (2004) also reported a somewhat later ripening time for 'Rajka' and 'Topaz' in the same region.

Table 1. Ripening time of the introduced apple cultivars

Cultivar	Ripening time			
	I	II	III	Mean
'Gala Must'	14th August	18th August	23rd August	18th August
'Rajka'	13rd September	19th September	23rd September	18th September
'Red Elstar'	29th August	3rd September	5th September	2nd September
'Topaz'	17th September	22nd September	25th September	21st September

The variance analysis of the fruit morphometric properties in the assessed cultivars reveals a significant impact made by the genotype on the parameters of fruit size, length of the stalk and number of seeds in the fruit. The year of trial also made a significant impact on all of the examined parameters, except the fruit shape index. The interaction effect cultvar/year made a significant impact on the fruit shape index, stalk length and number of seeds per fruit. The 'Gala Must' recorded the statistical largest fruit weight (157.56 g), whereas the lowest one was found in 'Rajka' (143.33 g) (Table 2). Based on the average fruit weight, all of the assessed cultivars can be classified in the category of cultivars with medium-sized fruits. The average fruit weight of 'Gala Must' is higher than the values reported by Milatović *et al*. (2009) for this cultivar (144 g). However, Rutkowski *et al*. (2005) pointed out that within the three years of study, the fruit weight of 'Gala Must' ranged between 144 g and 218 g. Blažek and Hlušičková (2007) report slightly higher values than the ones obtained for the fruit weight in our study, for 'Gala Must' (162.8 g), 'Elstar' (158.3 g), 'Rajka' (184 g) and 'Topaz' (164.6 g). According to Sosna (2014), based on the eleven-year long study, it was determined that the average fruit weight of 'Rajka' and 'Topaz' were 149 g and 135 g, respectively. The highest average fruit height was recorded in 'Gala Must' (61.33 mm), while the lowest one was found in 'Topaz' (54.21 mm). The 'Red Elstar' was characterised by the highest average fruit width (71.80 mm), whereas the smallest width was recorded in 'Rajka' (67.77 mm). Consequently, the value of the fruit shape index indicates the elongated (conical) shape of 'Gala Must' (0.88), i.e. the elongated-flattened shape of 'Rajka' and 'Red Elstar' (0.84 and 0.82, respectively), and the flattened shape of 'Topaz' (0.77). The 'Gala Must' recorded statistically largest length of the fruit stalk (30.77 mm), which is important from the aspect of the strength of bond between the fruit and the bearing branch, especially in the conditions

marked by the absence of chemical thinning of flowers/fruits. The largest number of seeds was found in the fruit of ‘Red Elstar’ (10.16). According to Uemura *et al*. (2001), this parameter has an impact on the final fruit set, as well as on the morphometric characteristics of the fruit.

Table 2. Fruit morphometric properties and yield of the introduced apple cultivars

Parameter		Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Fruit shape index	Stalk length (mm)	Number of seeds	Yield kg tree^{-1}	Yield t ha^{-1}
Cultivar (A)									
‘Gala Must’		157.56	61.33	69.94	0.88	30.77	7.26	24.63	61.58
‘Rajka’		143.33	57.07	67.77	0.84	24.69	8.10	20.94	52.35
‘Red Elstar’		149.42	58.62	71.80	0.82	25.44	9.16	12.42	31.05
‘Topaz’		145.36	54.21	70.46	0.77	28.78	10.16	21.52	53.79
Year (B)									
I		125.30	54.34	66.54	0.82	26.10	7.82	19.56	48.90
II		180.63	61.85	74.44	0.83	29.77	9.63	12.55	31.37
III		140.82	57.25	69.00	0.83	26.38	8.56	27.53	68.81
Cultivar × Year (A × B)									
‘Gala Must’	I	145.13	57.97	68.10	0.85	30.43	6.71	25.98	64.95
	II	170.87	65.03	73.07	0.89	32.93	7.85	13.53	33.82
	III	156.67	61.00	68.67	0.89	28.93	7.22	34.39	85.97
‘Rajka’	I	110.61	53.00	63.06	0.84	23.24	7.07	21.31	53.27
	II	197.38	61.56	73.56	0.83	26.42	8.77	10.44	26.10
	III	122.00	56.67	66.70	0.85	24.40	8.47	31.07	77.67
‘Red Elstar’	I	123.93	55.83	70.12	0.80	21.37	8.62	6.91	17.27
	II	169.77	60.83	74.20	0.82	29.40	9.59	9.35	23.37
	III	154.57	59.20	71.07	0.83	25.57	9.27	21.01	52.52
‘Topaz’	I	121.53	50.54	64.88	0.78	29.37	8.86	24.05	60.12
	II	184.49	59.97	76.93	0.78	30.33	12.32	16.87	42.17
	III	130.05	52.13	69.57	0.75	26.63	9.29	23.63	59.07
ANOVA									
A		**	**	*	*	**	**	**	**
B		**	**	**	ns	**	**	**	**
A × B		ns	ns	ns	*	*	*	ns	ns

The variance analysis revealed that the parameters of fruit chemical composition are determined by both the genotype and the year of trial. The interaction effect cultivar/year made a significant impact on the contents of sucrose and total acids. Cultivar ‘Topaz’ recorded the highest soluble solids content (14.86%), as well a total and invert sugars and sucrose (12.50%, 8.36% and 3.87%, resp.) (Table 3). The average content of soluble solids in ‘Topaz’ fruits was slightly higher compared to the values reported by Beber (2009) and Blažek and Hlušičková (2007), which fall within the range of 12−13.5%, i.e 13.1%. According to Soska and Tomala (2006), the content of soluble solids in ‘Gala’, ‘Elstar’, ‘Rajka’ and ‘Topaz’ is equable (13.5%, 14%, 13.8 % and 13.8%, resp.), whereas Blažek and Hlušičková (2007) reported this parameter at 13.6% for ‘Gala Must’ and 14.2% for ‘Elstar’. The highest content of total acids was found in ‘Topaz’ (0.54%), accompanied by the lowest pH value of the fruit juice (3.49%). Based on the content of total acids in the fruit, the ‘Gala Must’ (0.28%) can be classified within the group of sweet-fruit cultivars, whereas ‘Rajka’ (0.38%) belongs to the moderately sweet-frut cultivars, compared to ‘Red Elstar’ (0.52%) and ‘Topaz’ (0.54%), which belong to the group of cultivars with mildly acidic fruits.

Table 3. Fruit chemical properties of the introduced apple cultivars

Parameter		Soluble solids (%)	Sugar content (%)			Total acids (%)	Fruit juice pH value
			Total	Inverted	Sucrose		
Cultivar (A)							
‘Gala Must’		13.54	10.96	8.05	2.75	0.28	4.06
‘Rajka’		13.46	10.68	8.26	2.30	0.38	3.91
‘Red Elstar’		14.29	11.84	7.76	3.84	0.52	3.73
‘Topaz’		14.86	12.50	8.36	3.87	0.54	3.49
Year (B)							
I		14.68	11.98	8.44	3.36	0.50	3.70
II		13.09	10.63	7.39	3.07	0.37	3.91
III		13.62	11.30	7.87	3.26	0.37	3.92
Cultivar × Year (A × B)							
‘Gala Must’	I	14.70	11.95	9.08	2.73	0.32	4.02
	II	12.07	9.23	6.67	2.38	0.28	4.03
	III	13.87	11.70	8.39	3.14	0.25	4.12
‘Rajka’	I	14.20	11.28	8.51	2.63	0.47	3.74
	II	12.87	9.98	7.90	1.98	0.33	4.04
	III	13.30	10.78	8.38	2.29	0.34	3.96
‘Red Elstar’	I	15.20	12.62	8.35	4.05	0.69	3.55
	II	14.03	11.78	7.68	3.89	0.43	3.77
	III	13.66	11.01	7.24	3.58	0.42	3.87
‘Topaz’	I	14.63	12.05	7.81	4.01	0.53	3.47
	II	13.40	11.54	7.30	4.03	0.45	3.78
	III	13.63	11.70	7.48	4.01	0.47	3.72
ANOVA							
A		**	**	*	**	**	*
B		**	**	*	*	**	*
A × B		ns	ns	ns	*	**	ns

Yield of introduced apple cultivars

The yield of apple cultivars depends on the biological characteristics of the cultivar and rootstock, the age of the fruit tree, its health condition, proper choice of the polleniser cultivar, growing system, natural conditions and applied agro-technical measures. The variance analysis revealed that the yield per tree and unit of area were determined by the genotype and year of study (Table 2). The highest yield per tree and unit of area was recorded in ‘Gala Must’ (24.63 kg tree^{-1} and 61.58 t ha^{-1}), whereas the lowest one was obtained in ‘Red Elstar’ (12.42 kg tree^{-1} and 31.05 t ha^{-1}). Apart from ‘Gala Must’, a high cropping potential was found in ‘Topaz’ (21.52 kg tree^{-1} and 53.79 t ha^{-1}) and ‘Rajka’ (20.94 kg tree^{-1} and 52.35 t ha^{-1}). The yield of ‘Gala Must’, obtained in this study, is considerably higher than the values reported by Milatović *et al*. (2009), who also pointed to the higher productivity of the ‘Royal Gala’ sport, compared to the ‘Gala Must’ sport. At the same time, Rutkowski *et al*. (2005) stated a significantly higher productivity of the ‘Gala Must’, compared to the standard ‘Gala’ cultivar. The results of yield for ‘Topaz’ are in agreement with the data reported by Blažek and Hlušičková (2007), who pointed a very high productivity of this cultivar in the region of the Czech Republic. The same authors reported a higher productivity of ‘Topaz’, compared to ‘Elstar’, ‘Rajka’ and ‘Gala Must’. In our study, ‘Rajka’ showed a slightly lower yield compared to ‘Topaz’, which is in agreement with the results reported by Sosna (2014),

according to which ‘Topaz’ (130.6 kg tree^{-1}) recorded a higher cumulative yield sustained over a period of eleven years, compared to ‘Rajka’ (118.4 kg tree^{-1}).

Conclusion

Based on the study of the pomological properties and yield of the four introduced apple cultivars, it is possible to make the following conclusions:

The ‘Gala Must’ and ‘Red Elstar’ belong to the group of autumn apple cultivars, whereas the ‘Rajka’ and ‘Topaz’ belong to the winter apple cultivars;

Based on the morphometric features of the fruit, all of the cultivars can be classified within the group of medium-sized and elongated fruits (‘Gala Must’), i.e. the elongated-flattened (‘Rajka’ and ‘Red Elstar’) and flattened (‘Topaz’) fruits, whereas the largest average weight and height of fruit were determined in ‘Gala Must’;

The chemical composition parameters point to a very good quality of ‘Topaz’ fruits;

A high cropping potential was demonstrated by ‘Gala Must’, ‘Topaz’ and ‘Rajka’. The largest yield per tree and unit of area was obtained in ‘Gala Must’.

The examined intense-colouring sports of ‘Gala’ and ‘Elstar’, as well as scab resistant cultivars, were shown good results in the Čačak agro-ecological conditions, inferring that they can make a significant contribution to the advancement of the apple assortment structure in the Republic of Serbia. Based on the overall results, growers can be recommended to consider using ‘Gala Must’ as a dominant cultivar in the worldwide apple production, featuring outstanding quality and cropping potential, as well as ‘Topaz’, owing to its commercial significance as a scab resistant cultivar with outstanding productivity and fruit quality, suitable for integrated and organic farming.

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GRAIN YIELD AND STABILITY PARAMETERS FOR ZP MAIZE HYBRIDS GROWN IN CENTRAL SERBIA AND VOJVODINA DURING 2014

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Abstract

Breeding and selection of maize hybrids have been performed in the Maize Research Institute, Zemun Polje for the past 70 years. This study encompassed tests of 11 commercial ZP maize hybrids that were carried out in total of 50 locations in Vojvodina (25) and central Serbia (25) during 2014. The objective of the study was to estimate grain yield stability. The method after *Eberhart and Rusell* (1966) was applied in the estimation of yield stability. The average yield in Serbia varied from 9.469 t ha^{-1} (ZP 341) to 11.656 t ha^{-1} (ZP 606). Moreover, the hybrid ZP 606 was a record yielder in both Vojvodina (11.274 t ha^{-1}) and central Serbia (12.037 t ha^{-1}). On the other hand, the hybrid ZP 434 (b_i=0.987) was the most stable in all 50 locations, while the hybrids ZP 548 (b_i=0.980) and 427 (b_i=0.987) were the most stable in central Serbia and Vojvodina, respectively.

Key words: *maize, grain yield, yield stability*

Introduction

Maize, one of the most important field crops not only in Serbia but in the world, was domesticated some 7700 years ago in Central America. It represents a plant species that is evolutionary most developed in the entire plant kingdom (Glamočlija 2004). Maize is one of the leading species in the global agricultural production ranking high in the world economy. It ranks first according to total production quantity and grain yield per area unit and second according to harvested areas in the world (FAOstat, 2013). Approximately 1.2 million hectares are sown with maize in the Republic of Serbia. There are two large production regions in our country: central Serbia and Vojvodina. The main difference between these two regions is somewhat drier climate with a lower precipitation sum that is characteristic for central Serbia. Breeding and selection of maize hybrids have been performed in the Maize Research Institute, Zemun Polje for the past 70 years, with the aim to develop high and stable yielding hybrids that are adaptable to various environmental conditions of growth. Stability is the ability of a genotype to have always the uniform yield regardless of environmental effects (Becker, 1981). Adaptability (Finly and Wilkinson, 1963) is the ability of a variety to provide stable and high yield under different environmental conditions. One way to check hybrids is to test them in a greater number of locations. While testing the stability of hybrids, it is possible to happen that the highest yielding hybrids show below-average stability (Babic, 2006). The high stability of genotypes is one of the most desirable features as one of the main preconditions for the expansion of the same hybrids for cultivation in large areas (Singh and Choudhary, 1977). The greater number of locations under various environmental conditions is the greater probability of obtaining more reliable data related to grain yield stability is.

The aim of the present study was to observe productivity of commercial ZP maize hybrids and to establish grain yield stability in both, entire Serbia and its two main production regions, Vojvodina and central Serbia.

Materials and methods

A total of 11 commercial ZP maize hybrids of different FAO maturity groups (300-600) were studied in production trials set up in 50 locations in Vojvodina and central Serbia in 2014. Half of the locations were placed in Vojvodina, and the other half in central Serbia.

The elementary plot size amounted to 0.1 ha. Sowing and harvest were performed mechanically. The sowing density per hectare differed over FAO maturity groups and it ranged from 70,000 plants ha^{-1} (FAO 300-400) to 60,000 plants ha^{-1} (FAO 500-600). The grain yield ha^{-1} was calculated in t at 14% moisture.

The average yields were calculated cumulatively for all 50 locations and separately for Vojvodina and central Serbia, which are two distinct environments for maize cultivation. Stability parameters were estimated after method of Eberhart and Russell (1966) for both, 50 locations cumulatively, and Vojvodina and central Serbia separately.

Eberhart and Russell method is represented by the following equation:

$Y_{ij} = m + b_i I_j + d_{ij}$

Y_{ij} - the average yield of the i^{th} genotype in the j^{th} location

m - the average yield of the i^{th} genotype in all locations

b_i - standardised regression coefficient of the environment and genotype, genotype relationship to the environment

I_j - index of the environment as the average of all genotypes in the j^{th} location, reduced from the total average

d_{ij} - deviation from regression of the i^{th} genotype in the j^{th} site

The standardised regression coefficient (b_i) shows the response of genotypes to environment. When b_i=1, then the average adaptability and stability of the tested genotypes is considered uniform under the influence of favourable and poor environment conditions; when $b_i > 1$, genotype is considered stable and good only under favourable environment conditions, and when the $b_i < 1$, genotype is considered to perform better than the average in less favourable growing conditions and environments. All calculations were done in the Excel programme.

Results and discussion

According to obtained grain yields of maize, the year of 2014 was favourable. Due to above-average precipitation obtained yields were above the long-term average. Hybrids ZP 606 and ZP 560 over-yielded all remaining hybrids in all 50 locations (11.656 and 11.128 t ha^{-1}, respectively). On the other hand, the lowest average yield (9.496 t ha^{-1}) was recorded in the hybrid ZP 341. Based on values of the regression coefficient (b_i) it may be concluded that stability of all hybrids was satisfactory. The hybrid ZP 434 (b_i=0.987) was the most stable (b_i=0.987) of all hybrids in all 50 locations cumulatively. It is interesting to emphasise that the most yielding hybrid ZP 606 was at the same time the least stable (b_i=1.158) (Table 1).

Table 1. Grain yield of commercial ZP hybrids in production trials in all 50 locations in 2014

Hybrid	Grain Yield (t ha-1)	Rank according to grain yield	b_i	Rank according to b_i
ZP 341	9.469	11	0.890	8
ZP 366	9.841	9	0.848	10
ZP 388	10.140	8	0.913	6
ZP 427	10.249	6	0.912	7
ZP 434	9.603	10	0.987	1

ZP 548	10.214	7	1.082	5
ZP 555	10.570	5	1.124	9
ZP 560	11.128	2	1.058	4
ZP 600	10.988	3	1.013	2
ZP 606	11.656	1	1.158	11
ZP 666	10.957	4	1.015	3
Average	10.438	/	1	/

In the region of Vojvodina the highest average yields of 11.274 t ha^{-1} and 10.910 t ha^{-1} were recorded in the hybrids ZP 606 and ZP 560, respectively, while the lowest average yield (8.956 t ha^{-1}) was detected in the hybrid ZP 341. The values of the regression coefficient point out that the most stable hybrid in this growing region was ZP 427 (b_i=0.987), while the most yielding hybrid ZP 606 ranked second (b_i=1.047). It is interesting to state that the most stable hybrid ZP 434 in total, was the least stable (b_i=1.323) in the region of Vojvodina (Table 2).

Table 2. Grain yield and stability parameters of ZP hybrids in locations in the region of Vojvodina

Hybrid	Grain Yield (t ha^{-1})	Rank according to grain yield	b_i	Rank according to b_i
ZP 341	8.956	11	0.744	8
ZP 366	9.369	9	0.823	7
ZP 388	9.792	7	0.930	3
ZP 427	9.837	6	0.987	1
ZP 434	9.178	10	1.323	11
ZP 548	9.645	8	1.268	10
ZP 555	10.542	5	1.124	6
ZP 560	10.910	2	1.112	5
ZP 600	10.666	4	0.905	4
ZP 606	11.274	1	1.047	2
ZP 666	10.681	3	0.735	9
Average	10.077	/	1	/

The most yielding hybrids in central Serbia were ZP 606 (12.037 t ha^{-1}) and ZP 560 (11.345 t ha^{-1}), while the lowest yield was recorded in the hybrid ZP 341 (9.982 t ha^{-1}). The most stable hybrids in central Serbia were ZP 548 (b_i=0.980) and ZP 600 (b_i=1.058), while the least stable hybrid was ZP 555 (b_i=1.212). It should be also noted that the most yielding hybrid ZP 606 in all locations was very unstable in central Serbia, and therefore ranked next to the last (b_i=1.203), (Table 3)

Table 3. Grain yield and stability parameters of ZP hybrids in locations in central Serbia

Hybrid	Grain yield (t ha^{-1})	Rank according to grain yield	b_i	Rank according to b_i
ZP 341	9.982	11	0.892	5
ZP 366	10.312	9	0.817	9
ZP 388	10.488	8	0.904	4
ZP 427	10.661	6	0.868	7
ZP 434	10.029	10	0.861	8
ZP 548	10.783	5	0.980	1
ZP 555	10.598	7	1.212	11
ZP 560	11.345	2	1.079	3
ZP 600	11.311	3	1.058	2
ZP 606	12.037	1	1.203	10
ZP 666	11.234	4	1.126	6
Average	10.798	/	1	/

Conclusion

ZP hybrids in 2014 showed very good results in terms of both, grain yield and yield stability. The most yielding hybrid in total was ZP 606 (11.656 t ha^{-1}). This hybrid also over-yielded the remaining hybrids in Vojvodina and central Serbia (11.274 and 12.037 t ha^{-1}, respectively). The hybrid ZP 341 was the least yielding hybrid overall and in Vojvodina and central Serbia. Since the region of Vojvodina is to a certain extent more favourable for maize cultivation due to agro-ecological conditions, it was somewhat surprising that the average yield of all hybrids in central Serbia was higher by 721 t ha^{-1} than in Vojvodina. The precipitation sum was great in 2014 and therefore hybrids of later maturity groups were averagely more yielding than earlier maturing hybrids. Generally, ZP hybrids were very stable and their regression coefficient on the territory of entire Serbia varied from 0.85 to 1.16. The hybrid ZP 606 was most yielding in all locations, but at the same time, was the least stable (b_i=1.158), which is in accordance with results obtained by BABIĆ (2006). The hybrid ZP 434 was cumulatively the most stable (b_i=0.987). On the other hand, the hybrids ZP 427 (b_i=0.987) and ZP 548 (b_i=0.980) were the most stable in Vojvodina and central Serbia, respectively. Late maturing hybrids showed better results in 2014, which was characterised by a lot of precipitation. This result leads to a conclusion that later maturing hybrids will be more yielding and stable in more humid climate, while medium early maturity hybrids (FAO 300-400) will have better results in dry (arid) years, because their pheno-phases of flowering, ear formation and grain filling occur earlier in the year when soil moisture is still sufficient.

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OPTIMIZATION OF HERBS EXTRACTION FOR SOFT DRINK PRODUCTION

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Abstract

The polyphenolic compoundsnaturally present in medicinal and aromatic herbs have significant importance due to their bioactive and antioxidative properties, but sensorial, too. Herbs extracts obtained in this study have very pleasant odor, taste, and many bioactive compounds, therefore represent perfect base for soft drinks.

In this study, the aqueous extractions of 12 medicinal and aromatic plants were performed. The aim was to compare the content of polyphenolic compounds and antioxidant potential of obtained extracts. In order to optimize the extraction process, the influence of water temperature (room temperature, 60°C and 100°C) and extraction time (6h, 12h and 24h) were tested. The obtained extracts were characterized in their total polyphenolic content by Folin-Ciocalteu method whiles the antioxidant potentials were estimated by Ferric Reducing Ability of Plasma (FRAP) and 1,l-diphenyl-2-picryl hydrazyl (DPPH) method.

The results show statistically insignificant influence of extraction time. However, there were significant differences between samples obtained with different applied temperature. Also, the results indicate that the water extract of the medicinal and aromatic plants mixture is the great source of polyphenolic compounds with high antioxidant capacity.

These extracts of medicinal and aromatic herbs mixture could be applied in food industry, especially in the beverage industry for the development of natural products with high content of bioactive compounds.

Keywords: *herbs, water extraction, polyphenolic compounds, antioxidative properties, soft drinks*

Introduction

In recent years, research on phytochemicals, herbal food supplements and medicinal products has been intensified (Chen et al., 2007). Medicinal and aromatic plants contain a broad range of bioactive compounds such as alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones, oils (essential and fixed), flavors, fragrances and pigments. Also, rare compounds like furanocoumarins, hydroxycoumarins, napthoquinones, acylphloroglucinols and sterols are also distributed among these plants (Saroya, 2011). Many herbs are an excellent source of phenolic compounds which determine their considerable role in the prevention of various degenerative diseases (Capecka et al., 2005). Phenolic compounds, especially flavonoids and stilbenes, exhibit a number of bioactive effects, such as antioxidant, anti-inflammatory, antimicrobial, antiallergic, antithrombotic, anticancerogenic, antimutagenic, antiaging and vasodilatory activities.Except for a physiological role, phenolicshave a significant affect to sensorial properties of herb products, such as appearance, taste, mouth-feel, fragrance, astringency and bitternes. Phenolic compounds are generally considered as one of the main antioxidant sources in herbs, and antioxidant capacity is highly correlated with the total phenolic content(Jaganath et al., 2010).

Medicinal and aromatic plants in different forms (powders, extracts, etc.) are widely used in the food, pharmaceutical and cosmetics industries (Wang and Weller, 2006). Extracts are prepared by separating the soluble matter from plant tissues by application of a suitable solvent like alcohol, water or some organic solvent. In the case of extracts application in the food and beverage industry, the most suitable solvents are water and ethanol, because organic solvents could be source of harmful residues.The parameters which have the greatest influence on physicochemical and sensory properties of herb extracts are type of solvent, temperature of solvent, extraction time and techniques of extraction (Ong, 2004).

Herbal extracts are often used in soft drink production and first herbal drink is probably older than 5000 years (Whitehead, 2005). The aim of this study was to investigate the influence of water temperature and extraction time on the total phenolic content and antioxidant activity of aqueous extract of 12 medicinal and aromatic herbs. This extract is formulated as a base for soft drinks production. The combination and number of plants for the production of extract was chosen based on the sensory analysis of several different herbal extracts. Water was used as a solvent because it is only acceptable solvent for soft drink production.

Materials and methods

Extraction of herbs

The aqueous extraction of following 12 medicinal and aromatic plants was performed: peppermint (*Menthapiperita*), chamomile (*Matriciachamomilla*), wild thyme or creeping thyme(*Thymus serpyllum*), mountain germander (*Teucriummontanum*), winter savory (*Saturejamontana*),common yarrow(*Achilleamillefolium*), common sage (*Salvia officinalis*), mellisa (*Melissa officinalis*), centaurea (*Erythraeacentaurium*Pers.), wall germander (*Teucriumchamaedrys*), stinging nettle (*Urticadioica*) and wormwood (*Artemisia absinthium*). The extraction of plants was carried out in glass bottles which were placed on shaker in dark place at room temperature. Air-dried plants or its parts were cut into small pieces and placed into bottles. After that, the water was poured over the plants. The plant materials-water ratio was 1:8.Temperature of water was 20, 60 and 100°C, while extraction time was 6, 12 and 24 hours. After extraction, the water-plant mixture was pressed by laboratory hydraulic press and obtained extracts were filtered through 70 g/m^3 filter paper. Volume of final extracts was 7.4 liter; so, yield of production was 7.4 L of liquid extract per kg of dried herbs or 0.925 L of extract per liter of solvent (water).

Determination of dry matter of extracts was performed by drying of exactly weighed amount of fluid extract (approximately 5 g) in a Petri dish in a drying oven at 105°C until the mass becomes constant. After it was cooled in a desiccator for 30 minutes, the residue was weighed and the dry matter (%) was calculated as mass of residue/mass of fluid extract*100.

Determination of total phenolics

The amounts of total phenolics (TPC) in extracts were determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 0.5 mL of diluted extracts were mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu’s phenol reagent and allowed to react for 5 minutes. Two milliliters of sodium carbonate solution (75 g/L) was added to the mixture and then shaken. After 2 h of reaction at room temperature, the absorbance at 760 nm was measured. The calibration curve was prepared with gallic acid solution, and the results were expressed as milligrams of gallic acid equivalents per liter of sample (mg GAE/L).

DPPH radical-scavenging activity

DPPH radical-scavenging activity of extracts was estimated following the slightly modified procedure described by Kaneda et al. (Kaneda et al., 1995). Every diluted extract sample (0.2 mL) was added to the DPPH working solution (2.8 mL) (mixture of 1.86 x 10^{-4} mol/L DPPH

in ethanol and 0.1 M acetate buffer (pH 4.3) in ratio 2:1 (v/v)). The absorbance at 525 nm was measured after the solution had been allowed to stand in the dark for 60 min. The Trolox calibration curve was plotted as a function of the percentage of inhibition of DPPH radical. The results were expressed as millimoles of Troloxequvivalents per liter of sample (mM TE/L).

FRAP assay

The FRAP assay was performed according to the procedure previously described by Benzie and Strain, with some modification (Benzie and Strain, 1996). The FRAP reagent solution was made by mixing acetate buffering agent (pH=3.6), TPTZ (10 mM TPTZ solution in 40 mMHCl) and $FeCl_3$ x $6H_2O$ in volume ratio 10:1:1, respectively). All samples, standards and reagents were pre-incubated at 37°C. An aliquot of each diluted extract sample (0.1 mL) was mixed with distilled water (0.3 mL) and FRAP reagent (3 mL). After the reaction at 37°C for 40 min, the absorbance at 593 nm was measured. The calibration curve was prepared with Trolox solution and the results were expressed as millimoles of Troloxequvivalents per liter of sample (mM TE/L).

Statistical analysis

All experimentswere done in triplicate and obtained results are expressed as mean ± standard deviation (SD). Analysis of variance and significant differences among means were tested using ANOVA and Tuckey's HSD test ($p \leq 0.05$). Statistical analyses were performed with the statistical program Statistica 12.

Results and discussion

In this study, 9 different herb extracts was obtained. Table 1 shows an overview of the produced extracts, as well as the applied water temperature, extraction time and dry matter of extracts. The dry matter is a general parameter which measures the yield of extraction process and in obtained extracts was in the range from 0.93 to 1.93 %. The extraction yield was the highest in the extracts produced by extraction at 100°C, with no statistically significant differences between samples HE100/6, HE100/12 and HE100/24.The results indicate that increasing the extraction time is only relevant for the extraction at 20°C, because a longer extraction time did not influence the yield at 100°C and at 60°C after 12 hours. This means that the steady state between herbs and solvent (water)was reachedmore quickly at the higher temperature. Hinneburg and Neubert was obtained similar results during the extraction of buckwheat (*Fagopyrumesculentum*Moench) (Hinneburg and Neubert, 2005).

Table 1. Herb extracts overview

Extracts	Water temperature	Extraction time	Dry matter (%)
HE20/6	20	6	0.93±0.02
HE20/12	20	12	1.24±0.01
HE20/24	20	24	1.41±0.02
HE60/6	60	6	1.73±0.01
HE60/12	60	12	1.81±0.01
HE60/24	60	24	1.82±0.01
HE100/6	100	6	1.91±0.01
HE100/12	100	12	1.93±0.01
HE100/24	100	24	1.92±0.02

The total phenolic content and antioxidant properties of extracts are presented in table 2. Antioxidant capacity of samples was strongly and statistically significantly correlated with total phenolic content. All extracts was the very reach source of phenolic compounds, with the

total phenolic content in the range from 2048.82 to 4938.18 mg GAE/L. The results of the ANOVA test (table 3) indicates that observed factors (water temperature and extraction time) had a very significantly influence on the phenolic content and antioxidant activity of samples. The interaction of factors (temperature*extraction time) on the measured values was also very significant, which means that the impact of one factor depends on the level of the other factor.

Table 2. Total phenolic content and antioxidant properties of herb extracts

Extracts	TPC (mg GAE/L)	DPPH (mM TE/L)	FRAP (mM TE/L)
HE20/6	2048.82±10.10^{a}	14.62±0.13^{a}	13.49±0.09^{a}
HE20/12	2615.26±8.68^{b}	20.21±0.12^{b}	19.13±0.17^{b}
HE20/24	2892.48±23.76^{c}	21.79±0.14^{c}	21.11±0.14^{c}
HE60/6	4062.29±15.83^{d}	30.38±0.13^{d}	26.54±0.46^{d}
HE60/12	4340.80±21.99^{e}	33.85±0.07^{e}	30.50±0.31^{e}
HE60/24	4350.00±22.22^{e}	33.94±0.07^{e}	31.03±0.12^{e}
HE100/6	4726.50±9.42^{f}	36.16±0.08^{f}	34.53±0.16^{f}
HE100/12	4938.18±13.67^{g}	38.88±0.10^{g}	38.39±0.37^{g}
HE100/24	4901.24±4.13^{g}	38.79±0.09^{g}	38.65±0.06^{g}

Values represent means of triplicate determinations ± standard deviation. Different letters in same column denote a significant difference according Tukey's test, $p < 0.05$.

Table 3.The results of analysis of variance

Factors	TPC		DPPH		FRAP	
	F	p	F	p	F	p
Temperature	52645.28	0.00	77792.85	0.00	14147.31	0.00
Extraction time	1912.09	0.00	4749.12	0.00	1258.82	0.00
Temperature*extraction time	382.87	0.00	390.66	0.00	46.62	0.00

F - sample values applied test; p - level of significance (p≤0.05, difference is significant)

Water temperature had the very significant influence on the TPC of samples, where the best results were achieved with the temperature of 100°C. At 20°C, extraction time was important factor, and TPC and antioxidant activity was increased with prolongation of the process. However, after 12 hours at 60 and 100°C extraction time did not significantly effect on the TPC and antioxidant activity of extracts. Based on the results, it can be concluded that optimal parameters of this herbs mixture extraction is: water temperature 100°C and extraction time 12 hours.

Conclusions

The results obtained suggest that the extraction yield, TPC and antioxidant activities was the highest in the extracts produced by extraction at 100°C,with no statistically significant differences between samples HE100/12 and HE100/24. The steady state between herbs and solvent (water) was reached more quickly at the higher temperature, and because of that increasing the extraction time is only relevant for the extraction at 20°C. Extracts produced at 100°C are very rich source of phenolic compounds with considerable antioxidant activity. Therefore, these aqueous extracts are a very good base for development of different types of soft drinks with increased functional value.

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EFFECT OF NITROGEN FERTILIZER ON GRAIN WEIGHT PER SPIKE IN TRITICALE UNDER CONDITIONS OF CENTRAL SERBIA

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Abstract

Variability of grain weight spike^{-1} was studied in four triticale genotypes: 'KG-20', 'Tango', 'Odyssey' and 'Favorit', grown over a two-year period under four mineral nitrogen fertilizer treatments, 0, 40, 60 and 80 kg N ha^{-1}. The experiment was set up as a randomized block design in four replications. Averaged across genotypes, grain weight spike^{-1} increased with increasing nitrogen rate. On average, grain weight spike^{-1} in both growing seasons and under N treatment was highest in 'Favorit' and lowest in 'Tango'. Nitrogen fertilization made the largest contribution to the total phenotypic variance of grain weight spike^{-1}, cultivar was a considerably smaller contributor, and a low percentage was assigned to cultivar x nitrogen interaction. The results indicate that the value of the trait is significantly dependent on cultural practices.

Key words: *triticale, grain weight, spike, nitrogen*

Introduction

The combination of the high yield potential and good grain quality of wheat with the resistance/tolerance to biotic and abiotic stresses of rye resulted in the creation of a new small grain crop named Triticale (*x Triticosecale* Wittmack). Triticale breeding programs are mostly focused on major economic traits such as grain yield, biomass yield, nutritional value, earliness and grain filling percentage.

Triticale has moderate soil requirements and a good tolerance to low pH (about 5.0), with many genotypes showing better adaptation to acid soils compared to wheat cultivars (Oettler et al., 2000). As a new promising plant, triticale can also give satisfactory yields at high altitudes (Stošović et al., 2010). Moreover, triticale is highly tolerant of unfavourable weather conditions (Nožinić et al., 2009).

In recent years, triticale yields have ranged from 4 to 10 t ha^{-1}. Grain number per spike and grain weight per spike as direct yield components are determined by gene action under the influence of the environment (Mladenov et al., 1998; Malešević et al., 2010). Triticale is characterized by a high number of grains per spike and high grain weight per spike and per plant (Benbelkacem, 2002; Milovanović et al., 2006, Kondić et al., 2013).

To obtain high yields and good grain quality in triticale, special importance should be given to cultivar choice and proper cultural practices, particularly optimum nitrogen fertilization. Increasing nitrogen fertilizer rates generally increases grain yield in triticale (Stošović et al., 2010). However, high N rates may lead to moisture deficit stress due to intensive vegetative growth drawing on soil moisture reserves to the detriment of subsequent grain fill. Increased nitrogen application rates in small grains cause more intensive vegetative growth, more spikes m^{-2}, fewer grains per spike and variable grain weight (Paunović et al., 2006; Madić et al., 2013). The soil moisture x nitrogen level interaction is the main determinant of grain yield (Gonzales Ponce et al., 1993).

The objective of this study was to evaluate the effect of increasing mineral nitrogen rates applied during the growing season on grain weight per spike as a direct yield component in four triticale cultivars (genotypes).

Materials and methods

An experiment to analyze grain weight per spike in triticale was set up at the farm of the Secondary School of Agriculture, Kraljevo (the experimental field of the Faculty of Agronomy, Čačak) during the growing seasons 2012/13 and 2013/14. The soil at the experimental location had poor physical properties, an acid reaction (pH_{H2O} = 4.7), the content of humus 2.20%, readily available phosphorus 8.0 mg 100^{-1} g soil and potassium 14.0 mg 100^{-1} g soil.

Four triticale cultivars ’KG-20', 'Tango', 'Odyssey' and 'Favorit' were tested. The experiment involved an untreated control (0) and three mineral nitrogen (N) fertilization treatments: 40 kg N ha^{-1} (N40), 60 kg N ha^{-1} (N60) and 80 kg N ha^{-1} (N80). The N rates were applied through mineral fertilizer KAN (27% N) at the beginning of March in the early stage of intensive growth (jointing).

The experiment was laid out in a randomized block design with four replications and plot size of 5 x 1 m (5 m^2). Seeding was performed by a small mechanical drill in the second third of October at a spacing of 12 cm between rows and 3 cm within the row. At full maturity, 30 primary spikes were sampled from each plot and grain weight per spike was measured.

The results were subjected to a two-way analysis of variance (with cultivars and nitrogen as factors) using SPSS software (1995). Individual differences between means were evaluated by the LSD test.

Results and discussion

Grain weight per spike is a yield component that plays an important role in the productivity of triticale. It is a highly heritable quantitative trait and as such it is of great importance in the breeding process as well. Many authors have reported high grain weight per spike and per plant in triticale (Milovanović et al., 2006; Đekić, 2012; Kondić et al., 2013).

Grain weight per spike as a direct grain yield component is dependent on soil fertility, nitrogen nutrition, plant density, productive tillering, spike length and grain number per spike (Kovačević et al., 2006; Paunović et al., 2006; Izsaki et al., 2007). Grain weight per spike is mostly governed by grain number per spike, grain size and grain shape (Amin et al., 1995).

Table 1. Analysis of variance for grain weight per spike in triticale cultivars in 2013 and 2014

		Years					
		2013			2014		
			Variance components			Variance components	
Source of variation	df	F-test	σ^2	%	F-test	σ^2	%
Genotype	3	26.12**	$39.82x10^{-4}$	27.64	59.46**	$22.64x10^{-4}$	19.60
Nitrogen	3	89.66**	$91.61x10^{-4}$	63.60	138.73**	$75.36x10^{-4}$	65.24
Cultivar x nitrogen	9	1.97ns	$0.79x10^{-4}$	0.55	1.30	$3.20x10^{-4}$	2.72
Residual	48		$11.82x10^{-4}$	8.21		$14.30x10^{-4}$	12.38
Total	63						

** F –test significant at 0.01, ns F-test non-significant

The analysis of variance for grain weight per spike in triticale showed significant differences among genotypes, as well as among nitrogen application rates in both years (Table 1).

In both years, N accounted for the largest percentage of the total phenotypic variance of grain weight per spike, genotype made a considerably smaller contribution, and the lowest proportion was due to the genotype x nitrogen interaction. This suggests that the value of the trait is significantly dependent on cultural practices.

In the first year, the tested cultivars exhibited significant differences in grain weight per spike: it was highest in the lines ’Favorit’ and ’Odyssey’, followed by ’Tango’, and lowest in ’KG-

20’ (Tab. 2). Significant differences in grain weight per spike among the tested cultivars were also observed in the second year: the highest values were found in ’Favorit’, followed in decreasing order by ’Odyssey’, ’KG-20’ and ’Tango’ (Tab. 2). In the first year, increasing N application rates to 60 kg N ha^{-1} resulted in a significant increase in grain weight per spike (Tab. 2), in all cultivars.

In the second year, grain weight per spike increased in all genotypes with the N rate increasing to 80 kg ha^{-1}. Numerous studies have shown that increased N rates lead to a significant increase/decrease in grain weight per spike (Paunović et al., 2007 and 2008; Knežević et al., 2007).

Table 2. Grain weight per spike (g) in triticale cultivars under different nitrogen application rates (kg N ha^{-1}) in 2013 and 2014

		Year	
		2013	2014
Cultivar (A)	KG-20	1.02c	1.28c
	Tango	1.08b	1.21d
	Odyssey	1.12a	1.32b
	Favorit	1.14a	1.47a
Nitrogen (B)	0	0.97c	1.18d
	40	1.07b	1.25c
	60	1.15a	1.35b
	80	1.11a	1.39a
ANOVA	A	**	**
	B	**	**
	AxB	ns	ns

Means within line and nitrogen rate columns followed by the same lowercase letters are not significantly different at 95% according to the LSD-test

** F –test significant at 0.01, ns F –test non-significant

Moinuddin and Afridi (2008) found that the increase in mineral N fertilizer rates up to 200 kg N+ 40 kg P ha^{-1} had a positive effect on grain yield in triticale, whereas further increases caused a progressive decrease in the positive response (with overfertilization also leading to negative effects). Harmoney (2005) also reported a favorable response of triticale (grown for green forage) to increasing N rates up to a certain level.

Conclusions

Grain weight per spike significantly differed among the tested triticale cultivars in both years regardless of N application rates. The phenotypic variance components indicate that grain weight per spike is significantly dependent on cultural practices, since the total variance of the trait was mostly accounted for by mineral nitrogen fertilization, considerably less by cultivar and slightly by the cultivars x nitrogen interaction.

Increasing nitrogen application rates to 60 kg N ha^{-1} in the first year and to 80 kg N ha^{-1} in the second year led to an increase in grain weight per spike in all cultivars.

Significant differences among lines in both years across different nitrogen rates indicate that grain weight per spike is a genotype characteristic that may be used as a selection criterion for increased grain yield in triticale.

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DETECTION OF EPISTASIS FOR PLANT HEIGHT IN HEXAPLOID WHEAT (Triticum aestivum L.) USING GENERATION MEAN ANALYSIS

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Abstract

In order to estimate the gene effects in the inheritance of the plant height, five winter wheat genotypes (Pobeda, Renesansa, Sara, Partizanka and Pesma) were selected. The above mentioned genotypes were diallel crossed and F_1, F_2, and Fbc_1 were obtained. The gene effects were estimated on the basis of generation mean analysis, using an additive-dominant model with three and six-parameters (Mather and Jinks, 1982). The adequacy of the additive-dominance model with three-parameters was tested using the Scaling-test and Chi-square ($\chi 2$) test. In the most crosses the value of the dominant gene effect was more significant than additive. The three-parameter model was adequate for the three crossing combination: Pobeda/Pesma, Renesansa/Pesma and Sara/Pesma. In the remaining crossing combinations tests showed the presence of epistatic effect which suggested that three-parameter model was not adequate. The application of six-parameter models was fitted for explaining genetic variation for the plant height and indicated the presence of various epistatic effects. The inheritance of the plant height was influenced by additive×additive and dominance×dominance type of non-allelic interactions. Duplicate gene interactions were also seen functioning in controlling plant height in most crosses. The best fit model indicated a very similar gene effects which were presented by a model with six-parameters. Epistasis played a considerable role in controlling plant height of wheat which revealed that epistatic gene effect cannot be ignored in establishing a breeding program to improve wheat populations for this trait.

Keywords: *wheat, plant height, gene effects.*

Introduction

Considering the importance of wheat production in agriculture, the main aim in plant breeding programs is to create the wheat genotypes with a high genetic potential for the grain yield (Petrović et al., 2012). As an efficient breeding program requires understanding the nature, magnitude of gene effects and their contribution to the inheritance of the yield and yield components, in order to increase the genetic potential of wheat, it has been developed numerous different analysis methods. Among these, plant breeders often use generation mean analysis to get information about gene action which is controlling the traits. Generation mean analysis is a simple and useful technique for estimating gene effects for a polygenic trait. Since it has been reported that epistatic gene action is a non-trivial factor in the inheritance of plant characters (Goldringer et al., 1997; Erkul et al., 2010), the greatest contribution of this analysis lying in an ability to estimate epistatic gene effects such as additive×additive (i), dominance×dominance (j) and additive×dominance (l) effects (Singh and Singh, 1992). A large number of genetic studies have been made to investigate the genetic basis of yield and yield components of wheat. In most of different crosses were found that dominance effects and epistasis have been more important and predominant than additive effects for grain yield and its components (Erkul et al., 2010; Kumar et al., 2013). Plant height is one of several yield components and directly affects the yield. Plant height is largely governed by multiple gene factors. The importance of epistatic effects in controlling plant height was observed by

many researches (Ilker et al., 2009; Khattab et al., 2010; Ojaghi and Akhundova, 2010; Tonk et al., 2011). Therefore, the present study was carried out to obtain information about the types of gene effects for the plant height of different bread wheat genotypes.

Materials and methods

Five varieties of hexaploid wheat (*Triticum aestivum L.*), namely, Pobeda, Renesansa, Sara, Partizanka and Pesma were diallely crossed. It was obtained the five basic generations: parent cultivars (P_1, P_2), first and second filial generations (F_1, F_2) and first backcrosses (BC_1) of ten cross combinations (Pobeda/Renesansa, Pobeda/Sara, Pobeda/Partizanka, Pobeda/Pesma, Renesansa/Sara, Renesansa/Partizanka, Renesansa/Pesma, Sara/Partizanka, Sara/Pesma and Partizanka/Pesma). The five generations and their parent were sown in a randomized block design, with three replications, in three growing seasons (2009/2010, 2010/2011 and 2011/2012). The trial was set up on the trial field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The cultivars were sown in 2 m long rows with 20 cm of inter-row spacing and 10 cm spacing between plants in the row. The main sample consisted of 10 plants per replication. The plant height as a yield component was analyzed, at the stage of full maturity. The gene effects were estimated on the basis of Generation mean analysis, an additive-dominance model of three-parameters (Mather, 1949) and marked was according to Hayman (1960), where m = Mean effect, d = Additive gene effects, h = Dominance gene effects, i = Additive×additive epistatic effects, j = Additive×dominance epistatic effects and l = Dominance×dominance epistatic gene effects. The adequacy of the additive-dominance model could be tested in two ways, using the Scaling-test (A, B and C) and Chi-square ($\chi 2$) test. The simple genetic model (m, d and h) was applied when epistasis was absent, whereas in the presence of non-allelic interaction the analysis was proceeded to estimate the interaction types involved using the six-parameter genetic model i.e. (m, d, h, i, j and l) according to Mather and Jinks (1982). The type of epistasis was determined only when dominance (h) and dominance×dominance (l) effects were significant, when these effects had the same sign the effects were complementary, while different signs indicated duplicate epistasis (Kearsey and Pooni, 1996). The best-fit model was used for obtaining the degree of freedom. This model excludes gene effects which do not have a significant influence on the expression of the studied trait. The values of significant gene effects could be obtained using a matrix system.

Results and discussion

The additive-dominance model with three-parameter revealed that dominance effects (h) showed higher values than the additive effects (d), indicating that dominance gene effects play the major role in controlling the genetic variation of the plant height for all crosses (Table 1). The adequacy of the additive-dominance model was tested using Scaling test and Chi-square (χ^2) test. The results of these tests showed that the additive-dominance model, with three-parameter, was sufficient to explain genetic variation for the plant height for four crosses: Pobeda/Pesma, Renesansa/Partizanka, Renesansa/Pesma and Sara/Pesma. The results of the Scaling tests indicated that each of A, B or C were significant or highly significant for the plant height in the remaining six crosses: Pobeda/Renesansa, Pobeda/Sara, Pobeda/Partizanka, Renesansa/Sara, Sara/Partizanka and Partizanka/Pesma, which indicated the presence of non-allelic gene interaction for these crosses and revealed that additive-dominance model is inadequate for explaining the inheritance of plant height (Table 1).

Table 1. Estimated values of additive and dominance gene effects for the plant height of wheat using the three-parameter model

Cross combination					
Gene effect	Pobeda / Renesansa	Pobeda / Sara	Pobeda / Partizanka	Pobeda / Pesma	Renesansa / Sara
m	76.99	75.59	78.11	76.05	72.15
d	1.31	0.41	1.84	1.24	-1.30
h	3.79	7.95	3.97	4.11	1.52
Scaling test					
A	16.67**	6.16	12.37**	3.42	-21.28**
B	13.86**	5.73	7.34	0.28	-18.89**
C	7.92	41.13**	-3.42	3.20	1.84
χ^2 (3)	20.48**	15.45**	19.32**	0.80	70.14**
P (probability)	< 0.01	< 0.01	< 0.01	> 0.05	< 0.01
Cross combination					
Gene effect	Renesansa / Partizanka	Renesansa / Pesma	Sara / Partizanka	Sara / Pesma	Partizanka / Pesma
m	77.61	76.72	82.05	76.70	76.47
d	0.85	0.002	1.53	1.18	-1.03
h	-4.32	-4.09	0.84	5.06	4.68
Scaling test					
A	-7.37	5.08	15.58**	1.52	-16.96*
B	-9.57	5.40	10.98*	-1.19	-15.08*
C	2.87	0.77	52.62**	10.62	-8.67
χ^2 (3)	7.79	2.19	65.79**	2.41	16.85**
P (probability)	> 0.05	> 0.05	< 0.01	> 0.05	< 0.01

*Significant at 0.05, ** significant at 0.01

Therefore, the six-parameter model was applied and it was fitted for explaining genetic variation for plant height (Table 2). Using the six-parameter model it was confirmed the presence of significant epistatic effects in all cross combinations, except the three cross combinations: Pobeda/Pesma, Renesansa/Pesma and Sara/Pesma. Also, it was revealed that dominance effects (h) showed higher values than the additive effects (d), indicating that dominance gene effects play the major role in controlling the genetic variation of the plant height for all crosses.The importance of dominance effects in controlling of the plant height was observed by Petrović (1995), Sarker et al. (2007) and Fethi and Mohamed (2010). However, Farooq et al. (2010) and Tonk et al. (2011) reported that additive gene effects were more important for plant height in wheat. In the cross combination: Pobeda/Renesansa, Pobeda/Partizanka, Renesansa/Sara, Renesansa/Partizanka and Partizanka/Pesma, was observed that highly significant dominant effect (h), significant epistatic effects additive×additive and dominance×dominance epistatic effect contributed significantly to the inheritance of plant height. In these cross combination was detected duplicate type of non-allelic interaction, since dominance effects (h) and dominance×dominance epistatic effect (l) were significant and in opposite sign. In this case success of the selection would be affected negatively by these interactions. These results are in agreement with those obtained by Sarker et al. (2007) and Khattab et al. (2010), who also observed the presence duplicate type of non-allelic interaction in the inheritance of plant height. Contrary to these results, the presence of non-allelic gene interaction caused by complementary genes indicated Novoselović et al. (2004). In the cross combination Pobeda/Renesansa and Pobeda/Partizanka was observed the less favorable case of duplicate type of epistasis. The sign of the value of epistatic effects

dominance×dominance (l) were negative, which causes reducing the effects of dominant gene, which causes decreasing phenotypic expression of the trait. These results are less favorable for breeders, than if the values of dominance×dominance epistatic effects (l) were positive, as the crosses Renesansa/Sara, Renesansa/Partizanka and Partizanka/Pesma. In these crosses epistatic effect in fewer amounts masked the phenotypic expression of the trait. Also, the favorable side of these crossing combinations lies in the fact that in these crosses was significant epistatic effects additive×additive (i), which increases the ability to successfully selection more superior genotypes. Beside of that genetic effect that can be fixed (additive) was not significant, reported significant epistatic effects additive×additive (i), could be a result of some preferred interaction between the genes which are controlling this trait. However, the results obtained here, revealed the importance of epistatic effects in the inheritance of the plant height and should not be ignored in establishment a new breeding program to improve wheat genotypes for this trait. The preponderance of dominance and dominance×dominance epistatic effects for the plant height in the present study reveals that the expression of this trait was largely controlled by many genes with small effects and dominant in their action. When non-additive effects are larger than additive, the improvement of the trait needs intensive selection through later generation. The favorable situation was observed in the cross combinations Pobeda/Sara and Sara/Partizanka, considering in this crosses only epistasis additive×additive (i) significantly controlled the inheritance of the plant height and this effect additionally draws gene effects in the direction to the additivity. When additive effects are larger than the non-additive, it is suggested that selection in early segregating generations would be effective. The results of six-parameter model also indicated that epistasis wasn’t found in the inheritance of plant height at the crosses Pobeda/Pesma, Renesansa/Pesma and Sara/Pesma, which suggests that for this crosses an additive-dominance model was adequate, which greatly makes easier the selection for this trait, given the fact that the presence of epistasis complicated procedures for improving quality of traits.

Table 2. Estimated values of additive and dominance gene effects for the plant height of wheat using the six-parameter model

	Cross combination				
Gene effect	Pobeda / Renesansa	Pobeda / Sara	Pobeda / Partizanka	Pobeda / Pesma	Renesansa / Sara
m	53.12**	103.77**	53.69**	75.39**	117.95**
d	-1.41	-0.21	-2.51	-1.57	1.20
h	76.06**	-42.45	66.73**	8.05	-121.84**
i	22.61*	-29.24*	23.13*	0.50	-42.01**
j	2.82	0.43	5.02	3.14	-2.39
l	-53.14**	17.35	-42.84**	-4.20	82.18**
	Cross combination				
Gene effect	Renesansa / Partizanka	Renesansa / Pesma	Sara / Partizanka	Sara / Pesma	Partizanka / Pesma
m	98.05**	87.01*	103.10**	86.38**	101.77**
d	-1.10	-0.16	-2.30	-1.36	0.94
h	-60.04**	-33.48	-24.88	-15.48	-72.61**
i	-19.81*	-9.72	-26.05**	-10.28	-23.37*
j	2.21	0.32	4.60	2.71	-1.89
l	36.75*	20.21	-0.51	9.94	55.41**

*Significant at 0.05, ** significant at 0.01

Application of the best fit model is indicated in a very similar gene effects which were presented by a model with six parameters. Interactions caused by the presence of complementary genes, in which significant values (h) and (l) had the same sign, for the trait plant height in this experiment has not been determined (Table 3).

Table 3. Estimated values of gene effects for the plant height of wheat using the best fit model

Crosses	Gene effect	Estimated values	SE	t-test	Crosses	Gene effect	Estimated values	SE	t-test
Pobeda / Renesansa	m	52.97	12.8	4.14**	Renesansa / Partizanka	m	97.90	13.9	7.02**
	h	76.52	32.9	2.33*		h	-59.59	35.6	-1.67
	i	22.61	12.6	1.79		i	-19.82	13.8	-1.44
	l	-53.44	21.5	-2.48**		l	36.45	22.9	1.59
	χ^2 (2)	0,60				χ^2 (2)	0.30		
	P	>0.1				P	> 0.1		
Pobeda / Sara	m	82.2	2.0	41.80**	Renesansa / Pesma	m	75.06	1.3	56.63**
	i	-7.9	3.0	-2.60*		χ^2 (5)	1.90		
	χ^2 (4)	2.27				P	>0.1		
	P	>0.1							
Pobeda / Partizanka	m	53.09	13.9	3.80**	Sara / Partizanka	m	84.70	1.4	59.80**
	h	68.55	30.8	2.20*		i	-7.20	2.8	-2.50*
	i	23.13	13.8	1.70		χ^2 (4)	16.17**		
	l	53.09	13.9	3.80*		P	<0.01		
	χ^2 (2)	1.50							
	P	> 0.1							
Pobeda / Pesma	m	77.48	1.2	64.58**	Sara / Pesma	m	78.95	1.3	58.57**
	χ^2 (5)	2.2				χ^2 (5)	2.90		
	P	>0.1				P	> 0.1		
Renesansa / Sara	m	118.17	12.7	9.30**	Partizanka / Pesma	m	101.91	15.5	6.58**
	h	-122.48	29.7	-4.10**		h	-73.04	39.9	-1.83
	i	-42.01	12.5	-3.30**		i	-23.37	15.3	-1.53
	l	82.61	17.9	4.60**		l	55.69	25.6	2.17*
	χ^2 (2)	0.30				χ^2 (2)	0.10		
	P	>0.1				P	>0.1		

*Significant at 0.05, ** significant at 0.01

Conclusions

Based on the present findings it can be concluded that epistasis is a non-trivial factor and plays a considerable role in controlling plant height of wheat in crosses: Pobeda/Renesansa, Pobeda/Sara, Pobeda/Partizanka, Renesansa/Sara, Renesansa/Partizanka and Partizanka/Pesma. Dominance and dominance×dominance type of interaction effects are more important than additive or additive×additive interaction effects in the inheritance of plant height. The preponderance of dominance and dominance×dominance epistatic effects for the plant height in wheat reveals that the expression of this trait was largely controlled by many genes with small effects and dominant in their action. Considering that non-additive effects are larger than additive, the improvement of this trait needs intensive selection through later generation and could be much slower in a selection program.

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THE INHERITANCE OF PLANT HEIGHT IN HEXAPLOID WHEAT (Triticum aestivum L.)

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Abstract

Five winter wheat varieties (Pobeda, Renesansa, Sara, Partizanka and Pesma) have been selected for diallel crossing in order to estimate the mode of inheritance, gene effect and genetic variance components for the plant height in F_1 generation. The mode of inheritance was done on the basis of the significance of components of genetic variance and the regression analysis. The combining ability analysis indicated significant differences for the general (GCA) and specific (SCA) combining ability in the F_1 generation, which means that plant height had resulted from the genes with additive and non-additive impact. The best general combining ability was denoted in varieties Partizanka and Pesma and the best specific combining ability have shown in cross combination Partizanka/Pesma. The genetic components of variance, average degree of dominance and regression line indicated over-dominance in the inheritance of plant height.

Keywords: *wheat, plant height, diallel, regression.*

Introduction

Wheat is one of the most important crops in the world and its demand growing at approximately 2% per year, twice the current rate of gain in genetic yield potential (Reynolds et al., 2001). Since that grain yield is complex character influenced by many components, breeders normally use yield components to improve the grain yield, despite the fact that these components compensate each other in practice and increase in one cause a decrease in the other (Foroozanfar and Zeynali, 2013). Therefore, an efficient wheat improvement program requires an understanding of genetic mechanism involved in the expression of yield and yield components of plant material to be used in hybridization program (Shabbir et al., 2011). Plant breeders frequently use diallel analysis for testing a number of parental lines in all possible combinations. Diallel analysis is a suitable method for estimating of genetic parameters and provides early information on the genetic behaviour of these traits in the first generation (Farshadfar et al., 2012). Techniques for analyzing genotypes for all possible crosses include diallel analysis of variance, calculation of genetic components of variation, Vr/Wr regression analysis, as well as estimation general and specific combining ability effects and have been developed by Griffing (1956), Hayman (1954) and Mather and Jinks (1971, 1982). In the most of the diallel studies of wheat, genetic control of plant height, as one of several yield components of wheat, has been subject of numerous studies. Since that inheritance of plant height has complex nature, this question has been investigated in many its aspects (Zečević, 2005).

Therefore, the aim of this study was to obtain the information about inheritance of plant height in a 5×5 diallel cross of wheat. These results would be additional information in the selection of desirable parents for an effective breeding program to evolve new varieties of economic importance.

Materials and methods

Five winter wheat genotypes, (*Triticum aestivum L.*), Pobeda, Renesansa, Sara, Partizanka and Pesma, were crossed in all possible combinations following an 5×5 diallel mating system and it was obtained F_1 generation of progenies. The experiment was conducted at the trial field of the Institute of Field and Vegetable Crops in Novi Sad, according to random block design with three replications, in three growing seasons (2009/2010, 2010/2011 and 2011/2012). The cultivars were sown in 2 m long rows with 20 cm of inter-row spacing and 10 cm spacing between plants in the row. At the stage of full maturity, ten plants from each replication of hybrids and parents were selected randomly for recording data for plant height. Average values of three years trait analysis were used. Analysis of variance for parents and F_1 hybrids for the plant height was done according to Steel and Torrie (1980). General combining ability (GCA) and specific combining ability (SCA) was done following the Method 2 (parents and F_1 generation) Mathematical Model 1 of Griffing (1956). The regression analysis was conducted by the method of Mather and Jinks (1971). The components of genetic variance were analyzed following the models of Hayman (1954) and Jinks (1954).

Results and Discussion

The analysis of variance for plant height of wheat revealed highly significant differences among the replications and significant differences among genotypes (Table 1). Significant differences between replication indicated differences between years what was expected, given that the trial was conducted in three different growing seasons. Significant differences between genotypes suggested that the parents differ significantly and could be used efficiently for improvement of this trait. Using divergent genotypes in the crosses is preferable because selecting genetically divergent parents provides a wider genetic variability. These results are in accordance with earlier findings of Jadoon (2011) and Zeeshan et al. (2013).

Table 1. Analysis of variance for parents and F_1 hybrids for the plant height in a 5×5 diallel cross of bread wheat

Source of variance					F_t	
	DF	SS	MS	F	0.05	0.01
Replications	2	3743.09	1871.54	220.19**	3.32	5.39
Genotypes	14	332.06	23.72	2.79*	2.60	2.84
Error	28	237.99	8.50			
Total	44	4313.13				

[+]DF: Degree of Freedom, SS: Sum of Squares, MS: Mean Square, F: Level of Significance by the F test; Significant ($P < 0.05$), Highly significant ($P < 0.01$), Non-significant (ns)

Significant genotypic variation for this character was further partitioned into variation due to general combining ability (GCA) and specific combining ability (SCA) effects and presented in Table 2. The results of the analysis was indicated highly significant differences for the general (GCA) and significant differences for the specific (SCA) combining ability in the F_1 generation, meaning that plant height having resulted from the genes with additive and non-additive, i.e. dominant effects. The GCA/SCA ratio was equal and indicates that both kinds of gene effects were important in controlling the inheritance of the plant height of wheat. The present findings thus supported the results of Jadoon (2011); Farshadfar et al. (2013) and Zeeshan et al. (2013), which also showed the roles of both effects for plant height in wheat.

Table 2. Analysis of variance for combining ability for the plant height in a 5×5 diallel cross of wheat

Source of variance	DF	SS	MS	F	F_t 0.05	F_t 0.01
GCA	4	31.80	7.95	2.81**	2.69	4.02
SCA	10	78.88	7.89	2.78*	2.16	2.98
E	28	237.99	2.83			
GCA/SCA				1.01		

+GCA: General Combining Ability, SCA: Specific Combining Ability, E: Error; DF: Degree of Freedom, SS: Sum of Squares, MS: Mean Square, F: Level of Significance by the F test; Significant ($P < 0.05$), Highly significant ($P < 0.01$), Non-significant (ns)

Estimates of general combining ability (GCA) were presented in Table 3. The GCA estimates revealed that the tendency of the largest positive value of GCA effects were observed in genotypes Partizanka and Pesma, suggesting that these genotypes contain more genes with additive effects or additive×additive interaction effects and could be a good parent for this trait. Findings of current study were similar to findings of Zeehsan et al. (2013). Genotype Renesansa was the poorest general combiner with maximum negative but not significant GCA effects. Negative GCA effects were also observed in genotypes Pobeda and Sara. However, if in breeding program short stature behavior is preferred, therefore, negative combining ability effects are preferred for plant height. In that case, the most appropriate are genotypes with the highest negative values of combining ability.

Table 3. Estimates of GCA effects for the plant height in 5×5 diallel cross of bread wheat

Parents	GCA values	Rank	SE	LSD 0.05	LSD 0.01
Pobeda	-0.826 ns	4			
Renesansa	-1.216 ns	5	0.900	1.82	2.43
Sara	-0.127 ns	3			
Partizanka	1.084 ns	1			
Pesma	1.083 ns	2			

+GCA: General Combining Ability, SE: Standard Error; LSD: Least Significant Difference test; Significant ($P < 0.05$), Highly significant ($P < 0.01$), Non-significant (ns)

Estimates of specific combining ability (SCA) are given in Table 4. Crosses which displayed high SCA effects for the plant height were obtained from parents with various types of GCA effects (high x high, high x low and low x low). The highest positive significant SCA effect was exhibited by the cross Partizanka/Pesma (high x high general combiner). Tendency of higher, but not significant values of SCA effects was also observed in crosses Sara/Pesma (low x high), Renesansa/Sara (low x low), Pobeda/Sara (low x low) and Pobeda/Pesma (low x high). The highest negative SCA effects was shown by the cross Renesansa/Pesma, followed by crosses Renesansa/Partizanka, Sara/Partizanka and Pobeda/Partizanka. The SCA effect represents the dominance and epistatic interactions which do not contribute remarkably in the improvement of self-fertilizing crops as wheat, except for the exploitation of hybrid wheat where non-additive genetic variability could be utilized (Javaid et al., 2001). The most important are greater SCA effects in crosses which were involving both parents with high GCA, such as a cross Partizanka/Pesma, because they contain the additive×additive type of interaction which is fixable in later generations and can be used in future plant breeding. Greater SCA effects obtained in this cross, indicated the possibility of genetic improvement for plant height through pedigree selection and may be produce transgressive recombinants for this trait. Greater SCA effects obtained in crosses Sara/Pesma and Pobeda/Pesma which were involving one parent with high and other with low GCA, indicated the involvement of

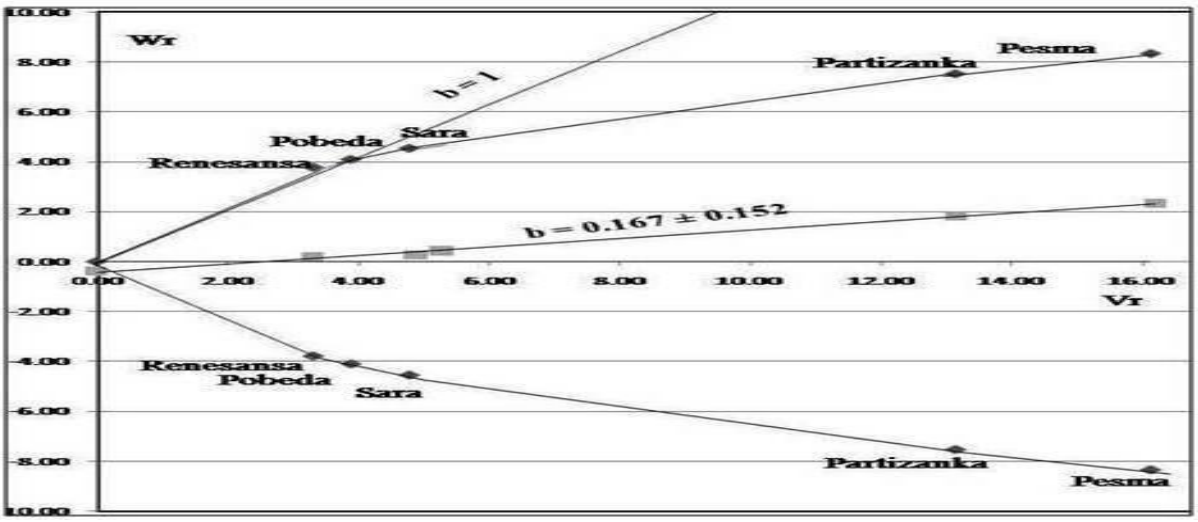
additive×dominance gene interaction in expression of this trait. Greater SCA effects obtained in crosses which were involving one parent with high GCA, was also reported by Ivanovska et al. (2003). Higher positive SCA effects exhibited by the crosses Renesansa/Sara and Pobeda/Sara which involving both parents with low general combiner, indicated the presence of non-allelic interaction at heterozygous loci. This interaction is unfixable, so it is suggested utilizing these crosses through single plant selection in the later generations (Hassan, 2004). Parents with high values of SCA are good combination of filial generation, which uses dominant gene action, but for wheat parents that show a high GCA are more important and are used in cases where the selection is performed in subsequent generations.

Table 4. Estimates of SCA effects for the plant height in a 5×5 diallel cross of bread wheat

							LSD	
Parents	Pobeda	Renesansa	Sara	Partizanka	Pesma	SE	0.05	0.01
Pobeda		0.462 ns	1.810 ns	-0.305 ns	1.357 ns			
Renesansa			2.033 ns	-2.714 ns	-3.752 ns	2.01	4.06	5.43
Sara				-0.867 ns	2.262 ns			
Partizanka					4.781*			

+SCA: Specific Combining Ability, SE: Standard Error; LSD: Least Significant Difference test; Significant (P < 0.05), Highly significant (P < 0.01), Non-significant (ns)

The regression analysis graph (Vr/Wr) for F_1 generations for plant height is presented in Figure 1. As it is seen in Figure 1, regression line intersected the Wr axis below the origin, indicated over-dominant inheritance of the plant height. Regression analysis (Vr/Wr) showed that regression coefficient in F_1 generation was significantly different from unity, suggesting the presence of non-allelic interaction for plant height. This result indicated the necessary to study effects of epistasis, as it may have a greater significance in some genotypes. Detection of epistasis suggested that variation for plant height of wheat was higher under polygenic control. The varieties Renesansa, Sara and Pobeda had higher dominant genes, but varieties Partizanka and Pesma, which were far away from the origin, had higher recessive genes. The arrays that correspond to parents were similarly distributed along the regression line and indicate that the parents were genetically divergent for the analysed trait (Fig. 1). These results suggest that selection would be difficult in the early generations for plant height due to over-dominance type of gene action. Similar results, which indicated over-dominance type of gene action, have been reported by Saleem et al. (2005) and Petrović et al. (2012).



b: Coefficient of Regression, Wr: Covariance, Vr: Variance

Figure 1.Vr/Wr regression analysis for the plant height of bread wheat

Genetics of the plant height, evaluated by calculation of the genetic components of variation is shown in Table 5. The analysis of the components of genetic variance indicated that dominant component (H_1 and H_2) was larger than additive (D). Unequal values of H_1 and H_2 suggested that positive and negative alleles were unequal among parent cultivars. The frequency of the dominant allele (u = 0.72) was greater than the frequency of recessive allele (v = 0.28), which is in agreement with the calculated F value (interaction additive×dominant effect), which is positive, as well as the value of $H_2/4H_1$. The average degree of dominance ($\sqrt{(H_1/D)}$ = 4.19) had greater values than 1, which indicated over-dominance type of gene action in the inheritance of the plant height, which was in accordance with the results obtained by regression analysis. The ratio of the total number of dominant against recessive alleles (K_D/K_R) was greater than 1, indicating the presence of more dominant alleles in inheritance of this trait. Preponderance of dominance effects suggested that selection for the trait in early generations may not be useful and it had to be delayed till late segregating generations. These results are in agreement with earlier findings of Zečević et al. (2005) and Jadoon (2011), while Minhas (2012) reported the predominant role of additive effects in affecting genetic mechanism of plant height and the involvement of partial dominance in the inheritance of this character.

Table 5. Genetic components of variation for the plant height of bread wheat

Components	Values	Components	Values
D	1.48	$H_2/4H_1$	0.20
H_1	25.88	u = p	0.72
H_2	20.72	v = q	0.28
F	1.17	$\sqrt{(H_1/D)}$	4.19
E	2.83	K_D/K_R	1.21

[+]D: Measures additive effect, H_1 and H_2: Measures dominance effect, F: Determines frequencies of dominant to recessive alleles in parents, E: Shows environment effect, $H_2/4H_1$: Determines proportion of genes with positive and negative effects in the parents, u: The values of dominant alleles, v: The value of recessive alleles, $\sqrt{(H_1/D)}$: The average degree of dominance, (K_D/K_R): Ratio of the total number of dominant against recessive alleles

Conclusion

The results revealed that there was significant genetic variation for the plant height of wheat among the genotypes and among the years. Significant GCA and SCA effects imply the role of both additive and non-additive gene actions in the genetic control of the trait. The largest value of positive GCA effects were observed in genotypes Partizanka and Pesma, suggesting that these genotypes contain more genes with additive effects or additive×additive interaction effects and could be a good parent for this trait. The highest positive significant SCA effect exhibited by the cross Partizanka/Pesma (high x high general combiner) indicated the possibility of genetic improvement for this trait through pedigree selection. The regression analysis in F_1 generations indicated over-dominant inheritance of the plant height, which was confirmed using the analysis of the components of the genetic variance. Regression coefficient was significantly different from unity, suggesting the presence of non-allelic interaction and indicated that variation for plant height of wheat is under polygenic control.

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THE EFFECT OF VETCH SEED SIZE ON THE SEED QUALITY AND ON SEEDLING VIGOR

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Abstract

Common vetch (*Vicia sativa*L.) in Serbia has the highest economic importance of the species *Vicia*. It is mostly cultivated jointly with cereals (oats, wheat, and triticale). It is rarely grown in pure culture due to flattening.

Producers have noticed that the market offer various sized seed. We decided to examine the connection between seed size with seed germination and vigor.

The paper presents two years results (2012 and 2013), of two cultivars of vetch seed (*Vicia sativa L*), and the three seed lots of each.

Seeds of each lot are divided into three fractions (C- small<3.5 mm; B - medium 3.5 to 5mm: A- large>5 mm. The germination and vigor of seeds are tested from all fractions (A, B, C).Germinated seeds were done in hot bed between the filter paper at 20°C in the dark; energy of germination was checkedon the fifth day, and the germination of the fourteenth day, in accordance with the ISTA rules.

Seed size had a significant impact both on the germination, and on the seedling vigor. The impact of the year, the party of seed and the varieties had no significant effect ($P \leq 0.05$). Between seed germination and seedling vigor was found high correlation (r).

Key words: *seed size, seed germination, seedling vigor, Vicia sativa*

Introduction

Common vetch (*Vicia sativa*) is a forage species with a long tradition of cultivation in Southeast Europe. The traditional way of use is the drying and haymaking. In recent years, often used for making haylage or silage, this has contributed to advances in the technology of conservation. In both modes of use,lodging is the problem in the phase of collecting. Therefore, it is grown mainly with cereals (wheat, oats, triticale), which increases the yield and partly solves the problem of collection but also the need to establish the optimum relationship between vetch and cereals (Mišković 1996; Yucel, andAvci, 2009; Karagićet al., 2011). For the projected ratio of legume andcereals, seed germination and seedling vigor (initial growth of stems and radicles) are of high importance.

On the other hand, the size of the seed is an important physical quality characteristic that often affect the initial growth of seedlings and in some cases on germination. For example, the leading agricultural crops (maize, rice) size of the seeds has been strongly influenced by genetics. Variation in seed size is due to the flow of nutrients to the seed in the mother plants therefore depending on the circumstances where the seed is located.

In literature there are a wide range of different effects of seed size on seed germination, however, these results differ substantially between species in the literature is insufficient data on the results of research of influence seed size on germination and seedling vigor beans(Kaydanand Yagmur, 2008;Kakhkiet al., 2008; Mut andAkay, 2010; Ambikaet al., 2014).The effect of vetch seed size on germination and vigor is not sufficiently explored, especially in Serbia

Therefore, the aim of this study was to determine the influence of seed size on germination and vigor, which would facilitate the choice of varieties to farmers according to size of seed. This would further determine the direction of breeders in creating new varieties.

Material and methods

In eastern Serbia was taken the seed of vetch (*Vicia sativa*),near Zaječar, 139 m asl, 43°58'N 22°18'E; with three lots (lot factor) of two cultivars (Neoplanta and Ovcepoljska, factor variety), for two years (2012 and 2013). Then the seeds are grouped into three fractions (C - small<3.5 mm; B - medium 3.5 to 5mm: A - large> 5 mm). Seed germination (%) was testedin the hot bed filter paper; 20°C - dark, fourteenth day was checked total seed germination (ISTA rules, 2003). By measuring each seedling (stem length – cm; radicle length – cm; fresh seedling weight- g) vigor was determined.

Statistical Analyses. Data were evaluated by analysis of variance (ANOVA) with Minitab 16.1.0 Statistics software package. When the *F*-test was significant, means were separated by Duncan's multiple range test at $p \leq 0.05$. An arcsine square-root transformation was performed on data percent. The correlation (r) between traits was established employing the Pearson's Correlation Test.

Results and discussion

Effect of variety, the seed lot and their interaction had no significant effect (F test; $P \geq 0.05$) on germination and vigor (Table 1). The reason is probably similar climatic conditions in 2012 and 2013 (table not shown) during critical phases of seed quality (filling, maturing and seed harvesting). Also, during both years in both varieties were applied the same breeding technology. The seed lot has no significant effect on germination in the grass species of *F. pratensis, F. arundinacea, F. rubra, D. glomerata* (Stanisavljević et al., 2013, 2014).

On the other hand, seed size showed a significant influence on seed germination and seedling vigor (Table 2). Between germination of large fractions (A 95%) and middle fractions (93% B), difference of 2% was not statistically significant ($P \geq 0.05$). Between middle-fractions (B) and fine fractions (C) difference of 3% was not statistically significant ($P \geq 0.05$). But, between the large (A) and fine fractions (C), achieved the difference of5%, which was statistically significant ($P \leq 0.05$).

The results ofbetter seed germination in larger seeds are consistent with the results obtained by Kaydan and Yagmur, 2008 with triticale; Kakhki et al. (2008)with wheat;Mut and Akay(2010)with oats and Sulewska et al.(2014)with maize. Overall, we should expect stronger initial seedling growth (vigor) because of higher feed into larger seed, which was necessary for the initial growth of seedlings.

In the fodder production, vigor (initial growth stems, radicles, and seedling weight) have high importance for the establishment of relations projected in grass-legume forage mixtures (Stanisavljević et al. 2011, 2013, 2014).

Table1. Results from analysis of variance (ANOVA) for: germination (G) and vigor,Seedling[stem(S), radicle (R), seedlingweight (SW)]. Sources of germination and variation include: year (Y), cultivar (C), lot (L) seed size (SS).

Source	G	Seedlingvigor		
		S	R	SB
Y	ns	ns	ns	ns
C	ns	ns	ns	ns
L	ns	ns	ns	ns
SS	*	***	**	**
Interactions				
Y× C	ns	ns	ns	ns
Y× C	ns	ns	ns	ns
Y× L	ns	ns	ns	ns
Y×SS	ns	ns	ns	ns
C ×L	ns	ns	ns	ns
C ×SS	ns	ns	ns	ns
L × SS	ns	ns	ns	ns
Y ×C× L	ns	ns	ns	ns
Y ×C× SS	ns	ns	ns	ns
Y ×L× SS	ns	ns	ns	ns
C × S × SS	ns	ns	ns	ns
Y ×C× L× SS	ns	ns	ns	ns

ns:Notsignificant*F* tests at the $P > 0.05$ level of significance.
*Significant *F* tests at the $P \leq 0.05$ level of significance.
**Significant *F* tests at the $P \leq 0.01$ level of significance.
***Significant *F* tests at the $P \leq 0.001$ level of significance.

In our studies, from seed fraction A, rising ofstems (24.52cm), radicles (15.24cm) and weight of seedlings (40.26g) are significantly stronger compared to seedlings resulting from seed fraction B (22.87cm stem, 2.14cm radicle, seedling weight 36.12g). Also, vigor in fraction B was significantly ($P \leq 0.05$) higher than seedlingvigor obtained from fraction C (20.23cm stem, 13.15cm radicle, seedling weight 31.97g); (Table 2). The results are consistent with general conclusion that, in agricultural plant species higher seed weight gives stronger seedling which is advantageous, especially in the initial rise (Ambika et al., 2014).

Table 2. Effect of seed size on germination and vigor (average of two years, two varieties and the three seed lots).

Seed size	Germination %	Seedling vigor		
		Stem length, cm	Radicle length, cm	Fresh seedling weight g
A	95±0.363a	24.52±0.399a	15.24±0.301a	40.26±0.471a
B	93±0.425 ab	22.87±0.402b	14,02±0.344b	36.12±0.524 b
C	90±0.492 b	20.23±0.443c	13,15±0.376c	31.97±0.528 c

Different letters within the same column note significant differences (Duncan's Multiple Range test, $P \leq 0.05$). Values are mean ± standard error of the mean

Table 3.Correlation coefficients (r) between the studied traits

Large	Germination (I)	Stem length (II)	Radicle length (III)	Seedling weight (IV)
		0.720***	0.630***	0.636***
	II	-	0.596***	0.603***
	III	-	-	0.567***
Medium	Germination (I)	0.648***	0.525***	0.503**
	II	-	542***	0.529***
	III	-	-	0.507**
Small	Germination (I)	0.504**	0.476**	0.482**
	II	-	0.447**	0.424*
	III	-	-	0,409*

Statistical significance level: *P≤ 0.05, ** *P*≤ 0.01, *** *P*≤ 0.001.

In the largest seed fractions (A) between germination and seedling vigor achieved the strongest correlation coefficient (r = 0.720 to 0.630, P ≤ 0.001;Table 3). Also, the germination of seed medium fraction (B) has achieved the same interdependence with vigor (r = 0.648 to 0.503; P ≤ 0.001). Between radicle length and seedling weight, correlation coefficient was not so high (r = 0.507; P ≤ 0.01).

Seed germination of fractions C, with all tested parameters of vigor, has also achieved positive, but lower dependence (r = 0.504 to 0.476; P ≤ 0.01). From this fractions, the lowest interdependence achieved between the weight of seedlings and radicle length (r = 0.409; P ≤ 0.05) and seedling weight and stemlength (r = 0.424, P ≤ 0.05;Table 3).

Conclusion

Large vetch seeds had a 5% higher germination than the small seeds and 2% higher germination than medium seeds. Large seedsachieved significantly stronger seedling vigor (stem 24.52cm,radicle 15.24cm, and weight of seedlings 40.26g) than medium seeds(stem 22.87cm, 14.2cm radicle, seedling weight of 36.12g.). Seedlings from small seedshad significantly lower vigor (stem of 20.23cm, radicle of 13.15cm, seedling weight of 31.97g.) thanmedium seeds.

Manufacturers prefer the larger seeds for better and stronger seedling vigor under field conditions. It is necessary to bear in mind the negative side of larger seeds, that it increases the sowing rate necessary for the realization of the optimum number of plants per unit area, which affects the price increase.

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GENETIC AND PHENOTYPIC CORRELATIONS AMONG STUDIED TRAITS OF DIFFERENT ALFALFA CULTIVARS

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Abstract

In this paper are studied 15 cultivars of alfalfa (*Medicago sativa* L.) of different geographical origins. In the studied alfalfa cultivars were tested for correlations of some important traits in 2007. year. Morphological traits were studied: height of plants (cm), number of stems, number of internodes per stem, green mass yield per cutting and the total yield of green mass (g). In the first cut were recorded medium strong positive correlation between the height and yield of green mass (r = 0.76 *). Heritability properties of plant height was very high in the first year (2006, h = 80.52) in the first cut, while in the second year of investigation (2007, h = 56.96), in the second cut. Estimation of heritability properties for green mass yield varied according to age, the highest value was obtained in the first cut 2006. year (h = 72.75), indicating a high share of genotype, while the lowest value was obtained also in the first cut in the 2007. year (h = 29.55). Breeding material possesses desirable traits for breeding new cultivars and with the choice of an appropriate model of breeding possible selection activities cultivars for specific purposes.

Key words: *alfalfa, cultivar, correlation, trait.*

Introduction

Alfalfa is a perennial and most important for multiple cuts fodder plant, which gives high yields and excellent quality forage in different environmental conditions (Đukić and Erić, 1995). Depending on the genotypes, characterized by high variability in forage yield (CV=28.6%) (Štrbanović, 2010). It is grown in the world over an area of 33 million hectares, while in Serbia represented an area of about 190.000 hectares. Areal of alfalfa cultivation on all continents covers more than 80 countries, from moderately cold to the tropics. The wide geographical distribution of alfalfa is conditioned by its great adaptability to different climatic and soil conditions (Julier *et al*., 1995). During of breeding certain traits that are controlled by a larger number of genes often lead to undesirable changes in the direction of some other important traits, which is due to mutual correlation in characteristics caused by the attachment of genes or pleiotropic effect (Šurlan-Momirović *et al*., 2005). It is therefore important to know the properties of interdependence traits, or in what way and to what extent one trait affects the other, which will allow easier identification of criteria and predict the course of breeding (Falconer and Mackay, 1996). Particular attention should be paid to genetic correlation coefficients that determine relationships between traits based only on genotype, without the influence of environmental factors.

Material and methods

In this paper studied 15 cultivars of alfalfa (*Medicago sativa* L.). Nine of selected cultivars of alfalfa were of the US origin and six of selected cultivars of alfalfa were of Europe origin (Table 1). The experiment was placed at the experimental field of the Institute for forage crops in Globoder at an altitude of 149 m. Experiment was placed on soil type alluvial sediment and is characterized by good physical - chemical properties, has a neutral pH value

(about 7), which is very favourable for growing alfalfa. Sowing of individual plants was performed manually in the spring, at the beginning of April 2005. at a distance of 60 to 60cm. The experiment was a randomized block design with four replications, with 60 plants per variety. In the course of performing experiments implemented a complete agricultural technology. Morphological traits were studied: height of plants (cm), number of stems, number of internodes per stem, green mass yield per cutting and the total yield of green mass (g). Based on the analysis of variance have been calculated components of variance studied traits.

Genetic (σ_g^2) and phenotypic (σ_f^2) components of variance:

$$\sigma_g^2 = \frac{MS_2 - MS_3}{r}\ ; \qquad \sigma_f^2 = \sigma_g^2 + \sigma_e^2\ ; \qquad \sigma_e^2 = MS_3$$

On the basis of the components of variance is calculated heritability in a broader sense (x_b^2):

$$h_b^2 = \frac{\sigma_g^2}{\sigma_f^2} \times 100$$

The coefficients of genetic and phenotypic variation (CVG and CVF) were calculated as follows:

$$CV_G = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100 \qquad CV_F = \frac{\sqrt{\sigma_f^2}}{\bar{X}} \times 100$$

The analysis of covariance examined characteristics of the studied alfalfa cultivars, calculated coefficients of genetic and phenotypic correlations.

- genetic correlation coefficient

$$r_{g_{xy}} = \frac{COV_{g_{xy}}}{\sqrt{\sigma_{g_x}^2 \times \sigma_{g_y}^2}}$$

- phenotype correlation coefficient

$$r_{f_{xy}} = \frac{COV_{f_{xy}}}{\sqrt{\sigma_{f_x}^2 \times \sigma_{f_y}^2}}$$

Table 1. The origin of the studied alfalfa genotypes (*Medicago sativa* L.)

Ordinal number	Cultivars	Origin
1	Mecca III	USA
2	Dynamic	USA
3	Pointer	USA
4	Weston	USA
5	DKA 50 – 18	USA
6	WL – 625 HQ	USA
7	Tru test	USA
8	Ameri stand	USA
9	Integrity	USA
10	K – 22	Serbia
11	K – 28	Serbia
12	Population Uzice	Serbia
13	Vali	Slovakia
14	Synteza 1	Slovakia
15	OS – 95	Croatia

Results and discussion

In the studied alfalfa, cultivars were tested for correlations of some important traits in 2007. year. Research in the first two cuts, which generate over 60% of the fodder and represent best performers and most importantly cuts alfalfa. Among individual characteristics were significant and highly significant correlation expressed genetic and phenotypic correlation coefficients (Tables 2. and 3.).

Table 2. Correlation coefficients of genetic and phenotypic traits studied alfalfa cultivars, 2007. the first cut.

Phenotype / Genotype	Height of plants	Number of internodes per stem	Number of stems	Green mass yield in the I cut	Total yield of green mass
Height of plants		0.57	- 0.57	0.76*	0.45
Number of internodes per stem	0.32		- 0.38	0.69	0.25
Number of stems	- 0.40	- 0.17		0.31	0.30
Green mass yield in the I cut	0.62	0.30	0.33		0.38
Total yield of green mass	0.46	0.17	0.29	0.31	

Table 3. Correlation coefficients of genetic and phenotypic traits studied alfalfa cultivars, 2007. the secund cut.

Phenotype / Genotype	Height of plants	Number of internodes per stem	Number of stems	Green mass yield in the I cut	Total yield of green mass
Height of plants		0.40	- 0.46	0.79*	0.44
Number of internodes per stem	0.21		0.02	- 0.33*	-0.22
Number of stems	- 0.14	0.24		0.49*	0.79*
Green mass yield in the II cut	0.65	- 0.35	0.44		0.32
Total yield of green mass	0.42	-0.15	0.57*	0.63	

In the first cut were recorded medium strong positive correlation between the height and yield of green mass (r = 0.76*). In the second cut were recorded medium strong positive correlation between the height and yield of green mass (r = 0.79*), number of stems and yield of green mass (r = 0.49*) and the number of stems and total yield of green mass (r = 0.79*). A

negative correlation was observed between the number of internodes per stem and yield of green mass (r = - 0.33*). For other traits calculated correlations were not significant. Radovic (2005) was found strong positive correlation between yield and number of stems (r = 0.66**), as well as medium strong correlation between the yield of green mass with the number of stems (r = 0.49*). Zonjić (1961) reported a high correlation between weight and plant height (r = 0.92**). In his research Zoric (1993) determined a strong correlation between the height of plants and yield of green mass (r = 0.69**), as well as number of stems and green mass yield (r = 0.78**). Strong positive correlations between height of plants and yield of green mass presented many authors in their research (Mijatovic, 1960, Zonjić, 1961, Ivanov, 1980, Hauptvogel, 1994, Katic, 2000). In examining the 36 alfalfa cultivars Varga et al., (1994), have determined a negative correlation between yield and number of internodes per stem (r = - 0.53**). The share of genetic variance is quite high for almost all the characters, which tells us that for the most part variation of the studied traits responsible genotype of the plant.

Plant height

Heritability properties of plant height was very high in the first year (2006) and reached h = 80.52 in the first cut, while in the second year of investigation (2007) reached h = 56.96, in the second cut. A very high share of genetic variation coefficient, which indicates that the value of phenotypic properties of plant height for the most part influenced by genotype.

In the estimation of heritability has a large influence plant density (Cristophe, 1973), so that with increasing plant density decreases heritability. Therefore, it can be concluded that the characteristics of plant height is highly heritable and that can be done on the basis of plant breeding level to improve the properties of yield (Table 4).

Table 4. The components of variance, heritability and coefficient of variation for plant height alfalfa cultivars studied from first to fourth cuttings in 2006. and 2007.

Trait	Height of plants							
	1. cut		2. cut		3. cut		4. cut	
Parameters	2006.	2007.	2006.	2007.	2006.	2007.	2006.	2007.
σ^2_g	101.8	66.24	12.51	66.24	11.56	26.03	27.44	40.06
σ^2_f	126.5	116.3	26.84	116.3	25.37	57.0	65.10	114.3
h^2_b(%)	80.52	56.95	46.61	56.96	45.57	45.67	42.15	35.05
CV_G(%)	9.68	9.92	4.58	9.92	4.72	7.67	9.53	8.93
CV_F (%)	10.78	13.14	6.71	13.15	6.98	11.36	14.67	15.09

Number of stems

The heritability of the characteristic number of stems per plant was quite high in both study years. The highest value of the year 2006. showed in the third cut (h = 62.47), while in the second year of investigation (2007), the highest value was shown in the first cut (h = 57.11). Genetic coefficient of variation is quite high in both studied years. The highest values showed in the first cuts in both years, and was 20.98% in the year 2006., while in the 2007. year showed slightly higher value 25.31%. All this indicates that the phenotypic characteristic of component number of stems under the strong influence of genetic components. Sikora, (1974) for this property states that the genetic variance in total phenotypic variance was 89%. The

high heredity of this trait suggests that it can be used as selection criteria in the plant yield, because there is a strong correlation between the number of stems and plant mass (Table 5).

Table 5. The components of variance, heritability and coefficient of variation for stem number of alfalfa cultivars studied from first to fourth cuttings in 2006. and 2007.

Trait	Number of stems							
	1. cut		2. cut		3. cut		4. cut	
Parameters	2006.	2007.	2006.	2007.	2006.	2007.	2006.	2007.
σ^2_g	84.43	229.8	79.59	189.6	139.8	136.2	109.3	80.14
σ^2_f	155.9	402.4	138.6	404.7	223.7	483.8	181.7	338.3
h^2_b(%)	54.14	57.11	57.41	46.85	62.47	33.74	60.18	23.69
CV_G(%)	20.98	25.31	15.93	16.30	18.79	15.81	19.29	16.13
CV_F (%)	28.51	33.49	21.02	23.82	23.73	27.22	24.87	33.15

Number of internodes per stem

Number of internodes per stem directly influences the increase of green mass and thus the quality of alfalfa, so that is a very important trait. The greatest value of this property as far as the heritability was found in the first cut of 2006. year (h = 58.81), while the lowest value recorded in the second cutting of 2007. year (h = 14.04) (Table 6).

Table 6. The components of variance, heritability and coefficient of variation for number of internodes per stem of alfalfa cultivars, studied from first to third cuttings in 2006. and 2007.

Trait	Number of internodes per stem					
	1. cut		2. cut		3. cut	
Parameters	2006.	2007.	2006.	2007.	2006.	2007.
σ^2_g	1.42	0.349	0.49	0.18	0.51	0.59
σ^2_f	2.41	2.244	1.37	1.27	0.99	1.48
h^2_b(%)	58.81	15.53	35.76	14.04	51.49	40.22
CV_G(%)	7.58	4.19	6.03	3.28	5.47	7.81
CV_F (%)	9.88	10.62	10.09	8.77	7.63	12.32

Green mass yield per cutting and the total yield of green mass

Estimation of heritability properties for green mass yield varied according to age, the highest value was obtained in the first cut of 2006. year (h = 72.75), indicating a high share of genotype, while the lowest value was obtained also in the first cut in the 2007. year (h = 29.55). Similar values as heritability demonstrated genetic coefficient of variation, which is in the first cut of 2006. was 21.45%, while in the first cut of 2007. was 12.03%. Such results can be explained by the strong influence of climate, drought and actually very high temperatures. Jacquard and Hanan (1970) found that the size of heritability for green mass yield depends on the conditions in which the estimate is done, an example density of the plants, so they get a higher percentage of heredity in rare plant density (h = 77) compared to the compacted plant density (h = 54). Due to the differences in heritability and the total yield of green mass, we can conclude that the heredity of green yield in under the increasing influence of environmental factors (Tables 7. and 8).

Table 7. The components of variance, heritability and coefficient of variation for green mass yield per cutting alfalfa cultivars studied from first to fourth cuttings in 2006. and 2007.

Trait	Green mass yield per cutting							
	1. cut		2. cut		3. cut		4. cut	
Parameters	2006.	2007.	2006.	2007.	2006.	2007.	2006.	2007.
σ^2_g	23862.5	4350.3	923.8	10838.6	1381.1	6217.4	949.9	4876.1
σ^2_f	32801.3	14721.5	3358.3	19588.9	3772.6	10926.9	2242.4	8303.8
h^2_b(%)	72.75	29.55	27.51	55.33	36.61	56.90	42.36	58.72
CV_G(%)	21.45	12.03	8.95	15.89	11.71	21.80	15.69	26.25
CV_F(%)	25.15	22.13	17.07	21.36	19.36	28.80	24.11	34.25

Table 8. The components of variance, heritability and coefficient of variation for total green mass yield alfalfa cultivars studied in 2006. and 2007.

Trait	Green mass yield - total	
Parameters	2006.	2007.
σ^2_g	35345.6	60509.9
σ^2_f	65936.1	177206.1
h^2_b(%)	53.61	34.15
CV_G(%)	11.95	13.86
CV_F(%)	16.32	23.71

Conclusion

Significant differences were found both between and within the studied alfalfa cultivars for most traits depending on cuts and years of research. The recorded positive correlations in the first and second cuts in the second year (2007) research. In the first cut were recorded medium strong positive correlation between the plant height and yield of green mass (r = 0.76*). Similar results positive correlations were obtained in the second cut. The negative correlation in the second cutting was found between the number of internodes per stem and yield of green mass (r = - 0.33*). Analyzing the components of variance is a very high share of genetic variance in total, which indicates that the impact of environmental factors on gene expression is very low, that is, for the most part responsible genotype variability. This confirms the very high values for the coefficients of heritability in a broad sense, ranging from 34.28% to 72.76% for the total dry matter yield. Breeding material possesses desirable traits for breeding new cultivars, and with the choice of an appropriate model of breeding, possible selection activities cultivars for specific purposes.

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GENOTYPIC AND PHENOTYPIC CORRELATION BETWEEN YIELD COMPONENTS IN S_1 AND HS PROGENIES OF AN F_3 MAIZE POPULATION

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Abstract

The main goal of the most maize breeding programs is to create superior inbred lines that could be used for getting newer, better commercial hybrids. It is necessary, by using suitable experimental methods, to investigate potential of every recently created population for possible adopting in long-term breeding programs. This paper presents the investigation of F_3 population 2002-30, created in the Department of Maize, Institute of Field and Vegetable Crops, Novi Sad. The investigation lasted for three years. At the first year, about 150 plants of the studied population were selfed and simultaneously crossed with the tester 1491x1496, in order to get complementary S_1 and HS progenies. After the harvest, 41 progenies of each type were selected by the random sample method. During the second and third year, trials were carried out in Aleksinac, Leskovac, and Kruševac, as three studied environments, in RCB design. Moderately strong and strong positive phenotypic correlation between yield components and grain yield was established, but between certain yield components negative correlation coefficients also were found. We found many significant positive, but also negative values of genotypic correlation coefficients; however, general tendencies were the same as for phenotypic correlation coefficients. Genotypic correlation coefficients of HS progenies were much greater than the phenotypic ones.

Key words: *maize, correlation, recurrent selection, yield components*.

Introduction

The main goal of the most breeding programs is to create superior inbred lines that could be used for getting newer, better commercial hybrids. It is necessary to evaluate, by suitable experimental design, potential of every new population for possible introduction in long-term breeding programs (Stojković, 2007). Choice of methods for dealing with a new synthetic population depends on level of favorable alleles, traits that selection is aimed for, their heritability, genotypic correlation between the most important traits, as well as on breeding goals, but a common characteristic of the all recurrent selection methods is selection and recombination of a lower number of superior progenies for next selection cycle (Deletić, 2003). Having in mind that studying intrinsic quantitative genetic parameters of every new population and their constant observing during selection process, in order to choose selection method and level, is hardly reachable in practice, every study of this type contributes to establishing general rules. Possible contribution of genes in achieving heterosis is usually determined by investigating half-sib progenies (i.e. test hybrids), while studying of S_1 progenies reveals additive effect of genes.

This study has been aimed to establish genetic and phenotypic interconnectedness among yield components, which is essential in considering selection for more than one trait, and for proper choice of selection methods. Selection for grain yield is a kind of selection for more than one trait, because it is a complex trait, highly dependent on yield components.

Material and methods

The object of this study was F_3 maize population 2002-30, created in the Department of Maize, Institute of Field and Vegetable Crops, Novi Sad (Serbia). During the first year of investigation about 150 plants of the population were selfed and simultaneously crossed with the tester 1491x1496, in order to get parallel S_1 and HS progenies. After the harvest, 41 progenies of each type (S_1 and HS) were selected for trials, by random sample method. During the second year a field trial was set in Aleksinac, and during the third year field trials were carried out in Leskovac and Kruševac (total three environments), in random complete block design with two replications, where the experimental plot area was 0.7x4.6 m. Number of plants per hectare amounted 62,112.

Grain yield is expressed as t/ha of grain with 14% of water, and the all plants of an elementary plot were used to determine grain yield and number of lodged plants. The other studied traits were analyzed on ten randomly chosen plants (i.e. ears) per progeny. A trait is expressed as the mean value of the investigated plants. Correlation coefficients, their standard errors and tests of significance have been calculated according to Falconer (1989), with the correction for negative values according to Deletić (2003).

Results and discussion

Correlation points to concordance degree of two or more traits. If genetic connection exists, selection for a trait will cause correlated response, i.e. change of the other traits mean value. For that reason, it is important to know this parameter for proper choice of selection methods.

Table 1. Phenotypic correlations (above diagonal) among the studied traits of S_1 progenies and their standard errors (below diagonal).

Traits	Grain yield	% lodged plants	Ear height	Plant height	Ear length	No of rows	Grains per row	1000 gr. mass
Grain yield	---	-0.25	0.63**	0.82**	0.46**	0.06	0.39**	0.32*
% lodged plants	0.31	---	-0.27	-0.46*	-0.38	-0.32	-0.22	-0.15
Ear height	0.07	0.28	---	0.84**	0.46**	0.44**	0.32*	0.27*
Plant height	0.03	0.20	0.03	---	0.45**	0.40**	0.31*	0.38**
Ear length	0.10	0.23	0.09	0.10	---	0.11	0.56**	0.36**
No of rows	0.18	0.26	0.10	0.11	0.16	---	0.17	-0.06
Grains per row	0.13	0.32	0.13	0.13	0.08	0.16	---	-0.15
1000 grain mass	0.13	0.32	0.13	0.11	0.11	0.17	0.16	---

* $p<0.05$, ** $p<0.01$

Grain yield of S_1 progenies was in strong, positive phenotypic correlation with ear height (0.63**) and plant height (0.82**), and in moderately strong, positive correlation with ear length (0.46**) and number of grains per row (0.39**). Significant, moderately strong, positive phenotypic correlation was found between grain yield and 1000 grain mass (0.32*). Moderately strong and strong, positive phenotypic correlation between certain yield components was established, but negative phenotypic correlation coefficients also were observed. The strongest negative phenotypic correlation connection was found between percent of root and stalk lodged plants and plant height (-0.46*) (table 1).

Table 2. Genotypic correlations (above diagonal) among the studied traits of S_1 progenies and their standard errors (below diagonal).

Traits	Grain yield	% lodged plants	Ear height	Plant height	Ear length	No of rows	Grains per row	1000 gr. mass
Grain yield	---	-0.56**	0.62**	0.85**	0.55**	0.07	0.55**	0.36**
% lodged plants	0.18	---	-0.56**	-0.73**	-0.55**	-0.50*	-0.46*	-0.02
Ear height	0.07	0.17	---	0.86**	0.48**	0.40**	0.31*	0.32*
Plant height	0.03	0.10	0.03	---	0.43**	0.37**	0.30*	0.41**
Ear length	0.09	0.17	0.09	0.10	---	0.16	0.60**	0.34**
No of rows	0.18	0.19	0.11	0.11	0.15	---	0.24	-0.09
Grains per row	0.09	0.22	0.13	0.13	0.08	0.14	---	-0.19
1000 grain mass	0.12	0.37	0.12	0.10	0.11	0.16	0.15	---

* p<0.05, ** p<0.01

Table 3. Phenotypic correlations (above diagonal) among the studied traits of HS progenies and their standard errors (below diagonal).

Traits	Grain yield	% lodged plants	Ear height	Plant height	Ear length	No of rows	Grains per row	1000 gr. mass
Grain yield	---	0.05	-0.13	0.50**	0.34	0.35*	0.23	-0.08
% lodged plants	0.42	---	0.28	0.42	0.08	0.25	0.25	-0.12
Ear height	0.31	0.35	---	0.38	0.09	-0.05	0.14	-0.12
Plant height	0.16	0.25	0.21	---	0.53**	0.25	0.44**	-0.09
Ear length	0.19	0.37	0.29	0.14	---	0.03	0.46**	0.09
No of rows	0.16	0.26	0.26	0.18	0.22	---	0.20	-0.54**
Grains per row	0.22	0.29	0.26	0.15	0.14	0.18	---	-0.34*
1000 grain mass	0.25	0.32	0.25	0.24	0.22	0.10	0.16	---

* p<0.05, ** p<0.01

Coefficients of genotypic correlation between grain yield of S_1 progenies and the other investigated traits were positive and highly significant, except number of grain rows per ear (0.07) and number of root and stalk lodged plants, where significant, moderately strong, negative correlation (-0.56**) was found. We also observed several significant positive and negative values of genotypic correlation, but general tendencies were the same as for phenotypic correlation coefficients (table 2).

Coefficients of phenotypic correlation between grain yield and the other traits of HS progenies were positive and moderately strong, significant for number of grain rows per ear (0.35*), and highly significant for plant height (0.50**). Moderately strong, positive phenotypic correlation was also observed between plant height and ear length (0.53**), plant height and number of grains per row (0.44**), and between ear length and number of grains per row (0.46**). Moderately strong, negative phenotypic correlation was observed between number of grain rows per ear and 1000 grain mass (-0.54**) and between number of grains per row and 1000 grain mass (-0.34*), while other coefficients of phenotypic correlation were not significant (table 3).

Table 4. Genotypic correlations (above diagonal) among the studied traits of HS progenies and their standard errors (below diagonal).

Traits	Grain yield	% lodged plants	Ear height	Plant height	Ear length	No of rows	Grains per row	1000 gr. mass
Grain yield	---	0.09	0.01	0.91**	0.51**	0.64**	0.32	-0.15
% lodged plants	0.41	---	-0.48	0.70**	0.11	-0.06	0.81**	-0.02
Ear height	0.35	0.25	---	0.60**	0.28	-0.16	0.18	-0.17
Plant height	0.03	0.13	0.14	---	0.69**	0.31	0.66**	-0.05
Ear length	0.15	0.36	0.23	0.09	---	0.01	0.37*	0.22
No of rows	0.09	0.33	0.23	0.17	0.23	---	0.20	-0.49**
Grains per row	0.19	0.07	0.25	0.09	0.16	0.18	---	-0.42**
1000 grain mass	0.23	0.36	0.24	0.25	0.19	0.11	0.14	---

* $p<0.05$, ** $p<0.01$

Genotypic correlation coefficients of HS progenies (table 4) were much greater than the phenotypic ones. Thus, correlative connection was highly significant, strong, between grain yield and plant height (0.91**), as well as number of grain rows per ear (0.64**), but moderately strong between grain yield and ear length (0.51**). Highly significant genotypic correlation was also observed between ear height and plant height, plant height and ear length, as well as between plant height and number of grains per row. Negative, highly significant correlative connection of moderate strength was found between number of grain rows per ear and 1000 grain mass (-0.49**), as well as between number of grains per row and 1000 grain mass (-0.42**).

Although there is a large number of previously reported data concerning correlative connection among various quantitative traits, especially grain yield and other traits, even a glance at some of them (Deletić, 1999; Deletić, 2003; Anđelković, 2000; Rošulj, 1999; Nastasić, 2001; Burak and Broccoli, 2001; Vasić et al., 2001; etc) reveals impossibility of a detailed comparison between them.

Conclusion

In S_1 progenies grain yield was in positive phenotypic correlation with plant height, ear length, number of grains per row and 1000 grain mass. Positive phenotypic correlation between certain yield components was established, but negative correlation coefficients also were found, e.g. between root and stalk lodged plants and plant height.

When genotypic correlation coefficients of S_1 progenies are observed, general tendencies were the same as for phenotypic correlation coefficients.

Phenotypic correlation coefficients of HS progenies were positive between grain yield and grain row number per ear, as well as between grain yield and plant height.

Genotypic correlation coefficients of HS progenies were much greater than the phenotypic ones.

Acknowledgement

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THE CRUDE PROTEIN CONTENT IN RED CLOVER GROWN ON DIFFERENT SOIL TYPES

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Abstract

Crude protein content is one indicator of red clover quality, which, among other things, is affected by the variability of soil properties. The aim of this study was to determine the effect of different soil types on the content of crude protein in red clover. The examination was conducted on six types of soil: chernozem, vertisol, eutric cambisol, humofluvisol, fluvisol and humogley (according to domestic soil classification). Sampling of plant material was carried out during May 2011, in the second year of red clover production. On the basis of these results it can be concluded that there are statistically significant differences in the content of crude protein between red clover plants, grown on different soil types. The highest protein content has red clover grown on chernozem (21.33%) and lowest on the fluvisol (14.33%). Soils, on which the protein content in red clover was higher, are characterized by good physical and chemical properties, a higher content of total nitrogen, primarily nitrogen fixation increased, as have the favorable conditions for microbial activity. This is primarily related to the pH value, because the greatest number of *Rhizobium* is in neutral and slightly alkaline soils. The expected results should be a guideline for the production practice, because the modeling of feed production, in different production conditions, raises the possibility of more intensive crop and livestock production.

Keywords*:* *red clover, crude protein, soil type*

Introduction

Protein content is one of the indicators of red clover quality. Variations in plant protein content are not conditioned only by plant species, but also by soil characteristics and applied growing technology (Živkov-Baloš et al., 1999).

Red clover is characterised by high protein content, which ranges from 186 to 264 g/kg of dry matter, depending on development stage and cultivar (Lugić et al., 2002; Lugić et al., 2006).

Red clover mainly satisfies its need for nitrogen (43-80%) by nitrogen fixation in a symbiotic association with the nodule bacteria *Rhizobium.* There are numerous factors that affect the number and size of nodules, as well as the quantity of fixed nitrogen: mineral fertilization, pH, temperature, moisture, soil aeration, etc. (Jakšić, 2015). Red clover averagely fixes 190 kg/ha per year, whilst it takes up around 3 kg on nitrogen to produce 100 kg of hay (Vučković, 1999).

Symbiotic microorganisms are more active in soils with favourable physical and chemical characteristics, which lead to higher protein content of red clover. It primarily relates to pH value, because the nodule bacteria are most abundant in neutral and slightly alkaline soils (Jakšić et al., 2013). In acid soils they are less abundant due to the lack of Ca and Al toxic effects (Govedarica & Jarak, 1995). In soils with pH below that 5.5, division of nodule bacteria slows down or nearly stops.

The aim of this paper was to study the effects of different soil types on crude protein content of red clover plants.

Materials and Methods

Studying effects of different soil types on crude protein content in red clover was conducted on six soil types (according to national classification): chernozem, vertisol, eutric cambisol, humofluvisol, fluvisol and humogley.

Soil samples were collected during May 2011 in second growing season of red clover. The testing was performed in Novi Sad, Žabari, Ratari, Kruševc, Šabac and Hetin in Serbia. Soil samples were in disturbed condition while taken by agrochemical probe from 0-30 cm depth. One representative soil sample consisted of 15-25 individual samples. Collected samples were air dried and milled by soil grinder to a particle size <2 mm according to SRPS/ISO 11464:2004. Total nitrogen content was determined by AOAC Official Method 972.43:2000, Microchemical Determination of Carbon, Hydrogen, and Nitrogen, Automated Method, in Official Methods of Analysis of AOAC International. The humus content was determined by Turin's method (JDPZ, Hemijske metode ispitivanja zemljišta, 1966). The content of calcium carbonate was determined volumetrically on Scheibler Calcimeter by method ISO 10693:1995. Active pH value was determined in soil-water suspension, while substitutional was determined with 1M KCl, potentiometrically according to method ISO 10390: 2005.

Plant material samples of red clover were collected in May, during flowering stage – first cutting. Fodder was cut manually at the beginning of flowering from an area of 10 m^2 by cutting plant at the height of 5 cm, in four repetitions. Nitrogen content was determined by AOAC Official Method 972.43:2000, Microchemical Determination of Carbon, Hydrogen, and Nitrogen, Automated Method, in Official Methods of Analysis of AOAC International. Crude protein content was determined indirectly via nitrogen content, multiplying with coefficient 6.25, which represents ratio of total nitrogen and crude proteins in legumes (Undersander *et al.*, 1993).

Results and Discussion

Basic chemical soil characteristics of all studied soils are shown in Table 1. According to average nitrogen content, chernozem and humogley were well provided, while the other types were medium provided with this nutrient element. Soils were acidic to slightly alkaline according to the soil pH, and non-carbonate to carbonate according to calcium carbonate content. Humus content characterised soils as low-humic to humic. Studied soil types had very low to optimal level of readily available phosphorus, as well as optimal to high level of readily available potassium.

Table 1. Basic chemical soil characteristics of studied soils

Soil type	pH-KCl	pH-H_2O	N (%)	$CaCO_3$ (%)	Humus (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)
Chernozem	7.38	8.11	0.21	5.47	2.81	15.95	29.33
Vertisol	5.88	6.89	0.16	0.48	2.45	3.93	23.15
Eutric cambisol	4.76	6.05	0.13	0.22	2.25	1.93	23.56
Fluvisol	5.53	6.58	0.15	0.07	2.30	3.96	16.36
Humofluvisol	6.52	7.55	0.15	3.75	2.11	14.66	23.17
Humogley	6.43	7.30	0.26	0.91	3.59	9.48	16.08

Trial results show significant effect of soil type on red clover protein content (Table 2). The highest average content was determined in tested forage plants from chernozem (21.33%), while the lowest in plant dry weight from fluvisol (14.33%). According to Džamić and Stevanović (2000), nitrogen content in full flowering of red clover amounts to 16.25%. Protein content in Vasiljević et al. (2011) of red clover from chernozem soil ranged from

17.59% to 18.32%. Marković et al. (2007) confirmed higher protein content of red clover in second cutting on alluvial soil - 19.74%.

Table 2. Protein content (%) in red clower

Soil type	Protein content (%)
Chernozem	21.33 a
Vertisol	17.54 d
Eutric cambisol	18.02 d
Humofluvisol	18.93 cd
Fluvisol	14.33 e
Humogley	20.59 ab
Average	**18.46**

Treatments marked with the same letter do not have statistically significant differences (Fisher’s exact test 0.05).

In the trial of Stević et al. (2007) on vertisol (pH value of 5.1), crude protein content in red clover averagely amounted to 17.90%.

Soils with higher nitrogen and dry weight protein content have higher total nitrogen content, and higher nitrogen fixation, due to favourable conditions for microbiological activity. It primarily relates to pH value, because the nodule bacteria are most abundant in neutral and slightly alkaline soils (Jakšić et al., 2013). In acid soils they are less abundant due to the lack of Ca and Al toxic effects (Govedarica & Jarak, 1995). In soils with pH below that 5.5, division of nodule bacteria slows down or nearly stops.

Grewal and Williams (2003) confirmed that significantly higher crude protein content was determined on treatments with liming, while Stout et al. (1997) stated that increase of crude proteins from 9.2% to 16.3% was achieved by application of lime material of 5 t/ha of CaO on soil with 5.1 pH value.

Correlation analysis was used to determine significant positive correlation between protein content in red clover and readily available potassium content in soil (r=0,58*), while regression analysis determined statistically significant average change of protein content in plants that occurs during change of readily available potassium content in soil (Figure 1). Regression coefficient showed that protein content in plants increased by 0.16% when the content of readily available potassium in the soil increased by one unit. Potassium has a positive effect on nodule bacteria growth, nodule formation and their functioning, as well as on plant growth, which results in higher nitrogen fixation (Divito and Sadras, 2014). In addition, K affects the metabolism of N by helping protein synthesis (Ubavić & Bogdanović, 1995).

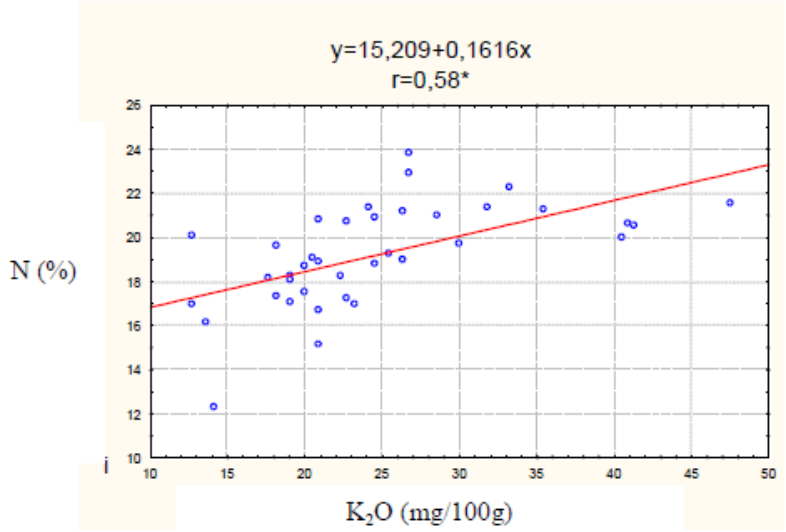

Figure 1. Dependence of protein content (%) in dry weight of red clover on readily available potassium content in the soil (mg/100g)

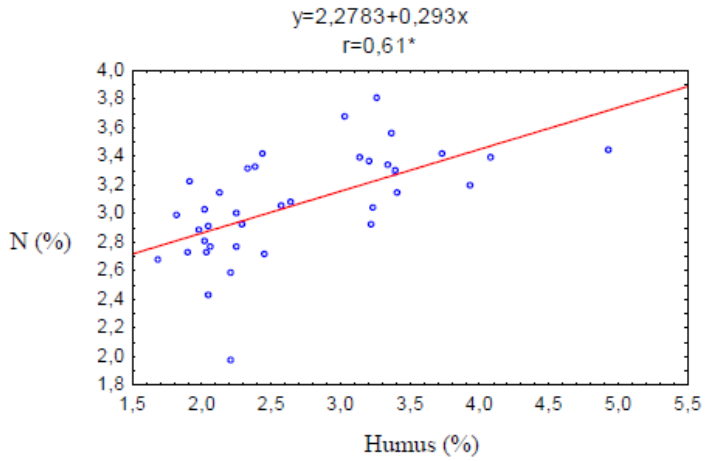

Figure 2. Dependence of nitrogen content (%) in dry weight of red clover on humus content (%) in the soil

Significant positive correlation between nitrogen content in red clover and humus content in the soil was determined (r=0.61*), while regression analysis was used to determine statistically significant average change in nitrogen content in plants, which occurs with change in humus content in the soil (Figure 2). Regression coefficient shows that the nitrogen content in plant increases by 0.29% when the humus content in the soil is increased by one unit. Presence of larger quantities of organic matter in the soil increases the content of elements necessary for plant nutrition, considering that their decomposition affects the nutrients to transform into mineral forms available to plants (Ubavić & Bogdanović, 1995). In addition, humus has a stimulating effect on biomass increase and macroelements uptake (Chen & Aviad, 1990).

Conclusions

The effect of soil type on crude protein content in the dry weight of red clover was significant. The largest average protein content was determined in studied forage plants grown on chernozem, while the lowest was in the dry weight from fluvisol.

More quality forage was obtained on soils with more favourable physical and chemical characteristics. Significant positive correlation between nitrogen content and humus content, as well as between proteins in red clover and readily available potassium in the soil was determined.

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CONDITION OF SOME PRIMARY METABOLITES IN WHEAT GRAINS DURING STORAGE

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Abstract

The effect of storage was studied on grain metabolites wheat cultivars selected in Serbia – Evropa, Pobeda and Renesansa. The samples (500 ± 5 g, 11–14% moisture) were placed into sealed plastic containers of the same volume, labelled and stored at a temperature of 25 ± 2 ^{o}C for 12 and 24 months. During the 12 and 24 months of storage, there was a decrease on average in lipids (3.55% and 5%), crude cellulose (16.65% and 21.77%) and an increase in total ash concentration (10.2% and 12.39%) and dry matter (10.01% and 10.31%).

Storage duration caused a significant decrease in lipids and cellulose, when compared to freshly harvested grains ($p \leq 0.05$). Ash levels increased significantly ($p \leq 0.01$) with storage duration. Prolonging of storage resulted in increased dry matter in grains of all the investigated cultivars ($p \leq 0.01$).

Key words: *wheat, storage, metabolites*

Introduction

It is important to store freshly harvested wheat grains before processing them for their future use. Wheat is an important cereal crop in people's diet worldwide. Due to its wide-spread use, wheat is the most commonly stored cereal crop. It is estimated that an average adult needs 150 kg of wheat per year. Storage duration negatively affects gluten levels (Rasheed et al., 2014). It is necessary to provide good storage conditions, in order to avoid undesired physical and chemical changes that can occur in grains during storage. For efficient storage, grain moisture and the temperature in the storage facility are very important (Rehman 2006; Rehman et al., 2002). Wheat grains with less than 12% of moisture can be stored for a longer period of time. Grains reduce germination ability over time, whereas their nutrition properties stay at a satisfactory level. Grains should be protected from rodents, insects and external weather conditions. An outer layer of grains can contain thousands of fungal spores and bacteria inactive at lower moisture levels. If grains have 13.5%-15% of moisture, fungal spores will develop more slowly. Grains with increased moisture levels, 16%-23%, provide almost ideal conditions for uninterrupted fungal development. Aerobic bacteria require 20% of moisture for growth and development. A combination of increased moisture and respiration of grains, fungi and bacteria results in releasing heat and evaporation of water that stimulate additional microbiotic activities (Nummer et al., 2013). Such biomass is no longer suitable for further use. All of this can have very serious economic consequences.

Materials and methods

Materials and storage conditions

The study investigated three commercial wheat cultivars – Pobeda, Renesansa and Evropa. Renesansa was bred at the Institute of Field and Vegetable Crops in Novi Sad and produced on the experimental field in Rimski šančevi, while Evropa and Pobeda were produced in Bajina Bašta. Pobeda is a mid-late winter wheat cultivar, with genetic potential for 11 tha^{-1}of

yield. It belongs to B1-A1 quality group and it is quite adaptive. Evropa is a bread wheat cultivar with good chemical composition and a high level of gluten. Renesansa is an early-mid-winter cultivar, with good chemical composition. It belongs to bread wheat cultivars, B1-A1 quality group. In 2012, all three cultivars were harvested and their grains were hulled.
Standard grain sampling techniques (ISO 24333:2009) were used. The samples were protected from insect infestations without using any chemicals. Then, the samples (500 ± 5 g, 11–14% moisture) were placed into sealed plastic containers of the same volume, labelled and stored at a temperature of 25 ± 2 ^{0}C for 12 and 24 months. The relative humidity of the storage rooms was recorded but not controlled. Storage temperature of 25 ± 2 ^{0}C was chosen to resemble the adverse temperature often found in storage facilities in south-eastern Europe. At the end of the previously defined period, three containers of the same sample were randomly taken, and their concentration was mixed and tested. Thus formed samples were then ground in a laboratory mill (1 mm mesh size).

Chemical properties

The level of moisture in the analysed samples was determined according to the AACC International (2000). Total lipids and total ash were determined according to Kaluđerski and Filipović (1998). The level of crude cellulose (crude fibre) was determined according to Veender, using ‘‘Fibertec 2010’’ and the instructions from the manual: Fibertec 2010 system, User Manual, 1009 9130/rev. 1.3, FOOS Tecator, Sweden. The samples were ground in a laboratory mill (Knifetec 1096). All the samples were tested in three replications, and the results were expressed on a dry matter basis.

Statistical analysis

Testing the differences between the genotypes and storage duration, as well as their interaction was conducted with the variance analysis of a two-factorial trial model (ANOVA), the F test for group comparisons and the Tukey’s HSD multiple comparison test for the risk level of 5% and 1% (Hadživuković, 1977; SAS Institute Inc., JMP, 1995). The effect of each factor and interactions between them was determined with the partial eta-squared coefficient, and classified according to Cohen’s classification (Cohen, 1988). All calculations were done in Data Analysis Software System (StatSoft. STATISTICA v. 10.0, 2010).

Results and discussion

The authors recorded different changes in nutritional properties that occurred over different periods of storage (12 and 24 months) in pre-defined conditions, at the temperature of 25± 2^{o}C. Storage duration and the temperature corresponded to the conditions commonly seen in storages in south-eastern Europe. Table 1 shows the levels of dry matter, ash and primary metabolites in the wheat grain samples stored at the constant temperature, for different periods of time. Dry matter in all the samples significantly decreased ($p < 0.01$) during 12-month and 24-month storage, by 10.01% and 10.31%, respectively. The obtained results are in accordance with a previous study (Rehman, 2006) that investigated changes in nutritional properties of maize, wheat and rice grains during storage. The total level of crude ash in all genotypes increased ($p < 0.01$) during storage, and significantly increased after 24 months. Data analysis showed the cultivars differed significantly in lipids and cellulose levels in grains.

Table 1. Investigated parameters, by different storage duration

Storage duration (months), B	Genotype, A	Contents (g kg^{-1})			
		Dry matter	Lipids	Ash	Cellulose
0	Evropa	871.17 $^{e\ a}$	21.93 $^{a\ b}$	14.97 $^{d\ c\ a}$	39.02 $^{a\ e}$
	Pobeda	872.83 $^{e\ b}$	20.84 ^{c}	15.95 $^{d\ b}$	38.27 $^{a\ d}$
	Renesansa	873.59 $^{e\ a}$	20.27 ^{c}	15.11 $^{d\ a}$	37.25 $^{b\ e}$
12	Evropa	891.60 $^{d\ ab\ c\ bc}$	20.85 ^{b}	15.94 $^{d\ c\ b\ c}$	33.03 $^{c\ d\ d\ b}$
	Pobeda	892.83 $^{d\ d}$	19.54 ^{d}	16.15 $^{d\ c}$	29.67 $^{e\ c\ c}$
	Renesansa	897.07 $^{b\ cd}$	18.38 ^{e}	16.73 $^{c\ b}$	28.72 $^{e\ d}$
24	Evropa	895.67 $^{b\ de}$	19.09 ^{d}	16.81 $^{c\ c\ b}$	30.02 $^{d\ a\ b\ d\ a}$
	Pobeda	893.70 $^{c\ e}$	17.97 ^{e}	17.19 $^{c\ c}$	28.43 $^{e\ b}$
	Renesansa	900.54 $^{a\ e}$	16.96 ^{f}	18.73 $^{a\ c}$	26.02 $^{f\ a}$
$HSD_{AB\ 0.05}$		3.66	0.95	1.63	1.86
$HSD_{AB\ 0.01}$		4.15	1.08	1.85	2.11

The grains of Evropa cultivar had significantly higher ($p \leq 0.01$) levels of lipids than the grains of the other two cultivars. Conversely, Pobeda and Renesansa had significantly higher ($p \leq 0.01$) levels of cellulose than Evropa. The results of previous studies (González-Torralba et al., 2013) on related topics indicate some changes that can affect negatively the quality of flour made from wheat grains stored for a longer period of time.

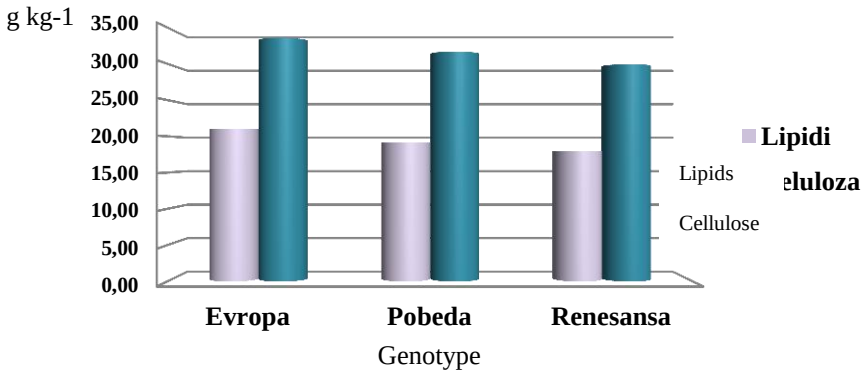

Figure 1. Lipids and cellulose in wheat grains, by genotype

When compared to freshly harvested samples, lipids and cellulose in grains of all the cultivars decreased significantly ($p \leq 0.05$) after 24-month storage (Figure 2). Previous studies (Rasheed et al., 2015) on monitoring the conditions of primary metabolites indicate there are some factors that can negatively affect the properties of mill products and the results of tests conducted during the process of making wheat bread.

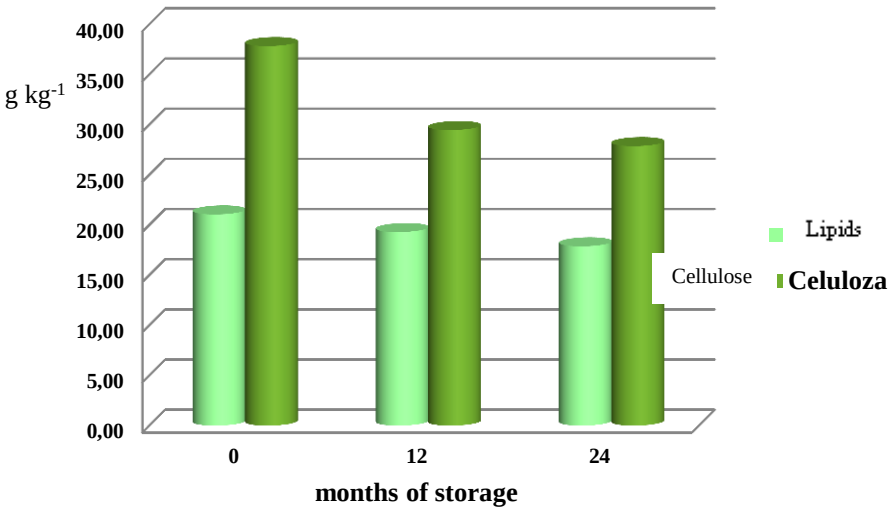

Figure 2. Lipids and cellulose in wheat grains, by storage duration (months)

In this study, ash levels in the grains were significantly ($p \leq 0.01$) affected by storage duration. Prolonged storage resulted in significantly increased ($p \leq 0.01$) ash levels (Figure 3).

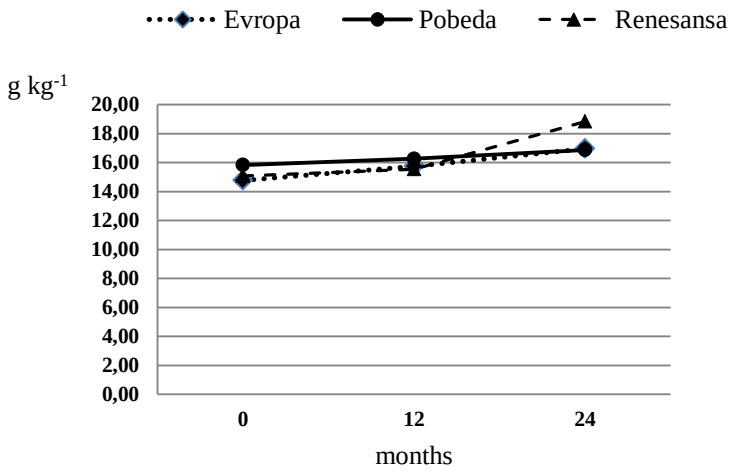

Figure 3. Storage duration and ash levels in the grains of the investigated wheat cultivars

These data are in line with the results of the previous study (Rakić et al., 2014) on storage of oat grains.

Conclusions

When it comes to lipids and crude cellulose, the nutritional properties of wheat grains changed during storage under the tested conditions. The levels of dry matter and total ash increased, when compared to the freshly harvested grains.

The results of this study show that wheat grains can be stored for 24 months under controlled storage conditions.

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VARYING OF AVERAGE YIELDS OF THE COMMERCIAL HYBRID ZPSC 341 OVER DIFFERENT LEVELS OF PERCENTAGE PARTICIPATION OF FERTILE PLANTS

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Abstract

The aim of the study was to determine the optimal ratio of male sterile (cms-S) to male fertile components of the hybrid ZPSC 341, in order to obtain the maximum yield in the commercial production. The present study encompassed observance of effects of different proportions of fertile to sterile plants on grain yield of the hybrid ZPSC 341. The three-replicate trial was set up according to the randomised block design. Twenty one mixtures with 0, 5, 10, …up to 100% of fertile plants mixed with the sterile variant of the hybrid ZPSC 341 were made. The original fertile hybrid ZPSC 341 was three times included into the trial as a check (hand-pollinated ZPSC 341, ZPSC 341 F1 and reciprocally crossed ZPSC 341), so as to control reliability of the trial. Yield, variations in yields and effects of fertile and sterile cytoplasm of the hybrid on yields were observed. The effect of the percentage of fertility, i.e. of sterile to fertile variant ratio on yield was determined. Gained results show that the highest average yield (16.071 t ha^{-1}) was achieved with 90% of fertility, in contrast to the hybrid with 30% of fertility in which the lowest yield (14.112 t ha^{-1}) was recorded.

Key words*: cytoplasmic male sterility ,maize, yield*

Introduction

Maize, due to its morphology, is a plant very suitable for the production of hybrid seed in large quantities, because hybridisation is relatively easily achieved by sowing parental components in alternate rows and by detasseling, i.e. removal of pollen-producing flowers (tassels) from female plants immediately after their exsertion. In such a way the following is achieved: pollen of solely male parents (which are not detasseled) circulates in the field, and hybrid seed is produced on female (detasseled) plants.

In order to achieve total hybridisation it is necessary to remove all tassels in female rows in due time (prior to pollen shed). This requires a great many workers, who have to be engaged in a relatively short period of time (10 to 30 days). Besides the provisions of detasselers it is necessary to provide appropriate control and super quality control of the work performed.

The machine cutting off tassels is the simplest solution to the problem of detasseling in maize hybrid seed production. Experiments with detasseling machines, cutters, had been performed by many researchers (Dungan and Wudworth, 1939; Borgeson, 1943; Kiesslbach, 1945; Bauman, 1959; Hunter et al., 1973 and others), and obtained results were summarised by Huey (1971) and Trifunović (1975). H u e y (1971) states that mechanical cutters of tassel are not usable under poor weather conditions, do not solve the problem of removing tassels on tillers and plants lagging in growth, and at the same time it is not possible to reduce the average number of leaves lost per plant bellow 2-3 even with the most careful work.

The possibility for an effective solution to the problem of detasseling in hybrid seed production has emerged with the discovery of cytoplasmic male sterility in maize. Using the

sterile male version of the female component completely eliminates the need for detasseling, then the number of workers needed for control tasks is minimised, production quality is improved and costs and associated risks are significantly reduced, and finally, in this way, the seed production becomes very attractive for producers.

The first description of male sterility was given by Rhoades (1931). Further investigations showed that sterility was caused by cytoplasmic factors.

Considering that the highest possible yields, with other favourable agronomic traits, are the principal aim of commercial production and in the light of increasingly strong competition in the seed maize market, it is necessary to evaluate the effect of maize sterility on grain yield of ZPSC 341, one of leading hybrids at the Maize Research Institute, Zemun Polje, and to determine the optimal ratio of sterile to fertile component for the need of commercial production of this hybrid.

Material and methods

In order to determine the optimal ratio of sterile to fertile variant of the hybrid ZPSC 341 in the commercial production, 21 mixtures with 0, 5, 10, up to 100% of fertile plants mixed with the sterile variant of the hybrid ZPSC 341 were made. The original fertile hybrid ZPSC 341 was three times included into the trial as a check (hand-pollinated ZPSC 341, ZPSC 341 F1 and reciprocally crossed ZPSC 341), in an attempt to control reliability of the trial.

Material and methods of setting up field trials

The three-replicate trial was set up according to the randomised block design in the location of Pančevo in 2013. The elementary plot consisted of two rows with 0.7-m inter-row distance, 10 hills per row, 0.37-m inter-hill distance and 2 plants per hill. The size of elementary plot amounted to 5.18 m².

The trial was set under conditions of dry-land farming. Sowing was performed at the optimum time (from April 5 to May 1). Standard maize cropping practices were applied.

The total number of plants, separately of fertile and sterile plants, was recorded for each elementary plot during the growing season when pollination was completed.

Harvest was done in the time of full maturity. The yield of fresh ear maize was measured at harvest for each hybrid per replicates and each elementary plot. The submitted sample consisting of five ears was measured with the technical balance in the laboratory.

Methods of experimental data processing

Statistical data processing encompassed the following: analysis of variance according to the randomised block design, regression and correlation analyses of grain yield and percentage of fertile plants in the hybrid ZPSC 341, so as to determine changes in grain yields in relation to the percentage ratio of sterile to fertile plants (according to Hadživuković, 1991).

Results and discussion

Table 1. Average yield and its variation interval for the check and different levels of fertility percentage

Ordinal number	% Fertility	Average yield (t ha^{-1})	95-% interval of confidence for mean yield	
			Lower limit	Upper limit
1	Hand-pollinated ZP341	14.980	13.637	16.322
2	ZP341F1	13.952	13.744	14.159
3	ZP341Rec.	14.718	12.232	17.204
4	0%	14.260	12.369	16.150
5	5%	14.590	13.687	15.494
6	10%	14.235	13.328	15.142
7	15%	14.294	12.489	16.098
8	20%	15.137	14.045	16.230
9	25%	14.903	12.764	17.043
10	30%	14.112	12.743	15.481
11	35%	14.669	14.286	15.051
12	40%	14.515	13.711	15.320
13	45%	14.435	12.901	15.970
14	50%	14.503	10.921	18.085
15	55%	14.916	14.562	15.270
16	60%	15.279	13.604	16.953
17	65%	15.237	14.029	16.446
18	70%	14.713	11.857	17.569
19	75%	14.804	11.850	17.758
20	80%	14.925	14.632	15.217
21	85%	15.151	12.749	17.552
22	90%	16.071	15.625	16.517
23	95%	15.435	14.694	16.175
24	100%	15.269	13.678	16.860

Table 1 shows that the most yielding hybrid (16.071t ha^{-1}) had 90% of fertile plants, while the hybrid ZPSC 341F1 was the least yielding (13.952 t ha^{-1}).

According to the stated, it may be concluded that edaphic and climatic conditions in the given location had a crucial effect.

The conclusion made by comparison of average yields (14.796 t ha^{-1}) obtained in the location of Pančevo and yields of hybrids ZP 360 and ZP 434 (14.160 and 14.260 t ha^{-1}) recorded by Videnović et al. (2000) in the location of Sombor, is that achieved average yields were approximately equal.

Furthermore, studies of the most recent the 5th and the 6th generation of ZP hybrids carried out by Jovanović et al. (2007) show that the highest yields in Serbia were recorded in the following hybrids: ZP 684 (9.50 t ha^{-1}), ZP 544 (9.23 t ha^{-1}) and ZP 434 (9.21 t ha^{-1}). The hybrids ZP 341 (10.02 t ha^{-1}) and ZP 434 (9.50 t ha^{-1}) were the most yielding in the region of Banat, while the highest yield in the region of Srem was achieved with the hybrid ZP 434 (11.34 t ha^{-1}). Moreover, based on long-term studies on medium late maturity hybrids with a

shorter growing season carried out by the group of researches, it was concluded that given hybrids had significantly lower grain moisture content (16-18%).
Based on everything stated, it may be concluded that the 5th generation of ZP hybrids (FAO 300-400) expressed exceptional yielding and yield stability. Additionally, these hybrids are characterised by a shorter growing period and significantly lower grain moisture at harvest, which is great advantage due to reduced costs of maize drying and storage.
Results presented in Table 2 point out that different ratios of sterile to fertile components in the seed mixture used in sowing do not significantly affect achieved yields (r=0.390).

Table 2. Correlation coefficient of yield and fertility percentage

Ordinal number	Location	r_{xy}
1	Pančevo	0.390

Furthermore, we were not able to determine a relative importance of each independent variable for depended variable - yield (Table 3). Insignificant effects of various ratios of fertile to sterile components are noticeable through low regression coefficients (ß). Their contribution to the changes in yields amounts to only 15.2 % (R^2).

Tabela 3. Values of parameters of squares regression model and coefficient of determination

Location	β_0	β_1	β_2	R^2
Pančevo	-4E-05X^2	0.0118X	10.049	0.152

According to the coefficient of determination, a small percentage dependence can be observed, pointing to the fact that a high percentage of variance affecting yield variation was not encompassed.
The coefficient of determination for the location of Pančevo (0.152) is presented in Figure 1.

Figure 1. Calculated squares regression equation for the location of Pančevo.

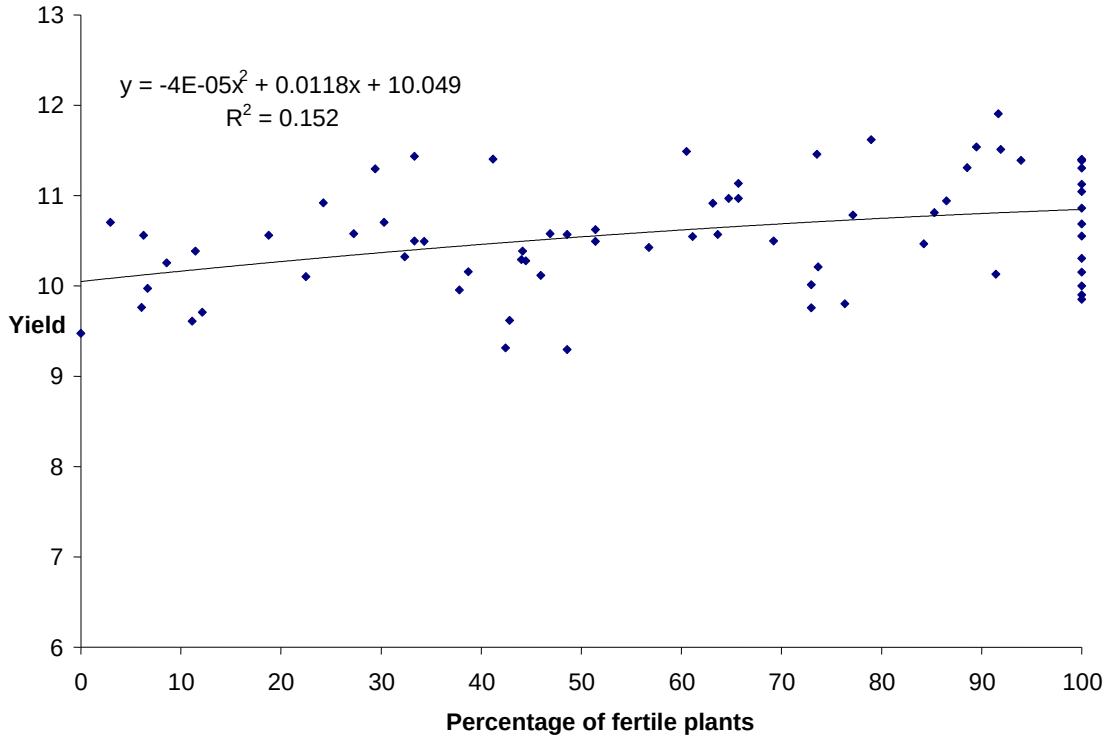

Figure 1 does not show regularity of effects of percentage of fertile and sterile plants on yields, which points out to the possibility of their independence.

Conclusion

Issues related to the commercial seed production of the hybrid ZPSC 341 and effects of different percentages of fertile and sterile plants on yield of this hybrid were observed in this study.

According to obtained results, the following may be concluded:

The location statistically significantly affected maize grain yield;
Environmental conditions significantly affected yield variation
The highest recorded yield amounted to 16.071 t ha^{-1}
The lowest recorded yield amounted to 13.952 t ha^{-1}
The most favourable ratio of fertile to sterile variant was 90% of fertile to 10% of sterile plants;
The least favourable ratio was with the participation of 30% of fertile plants (14.112 t ha^{-1})
Although correlation coefficients were positive, there was no statistical significance of yield and percentages of fertile and sterile plants.

Although obtained results do not show the optimal ratio of sterile to fertile variants of the hybrid ZPSC 341 for its commercial production, there are sufficient reasons to assume that the previously applied 75% to 25% ratio of fertile to sterile variants is the optimal one for the commercial production of the hybrid ZPSC 341.

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INFLUENCE OF FERTILIZATION AND TILLAGE ON MAIZE GRAIN QUALITY TRAITS AND YIELD

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Abstract

The effects of fertilization and tillage on grain quality trait and yield of maize hybrid was evaluated. An experiment was laid out in split plot design, having three replication, with following treatments: no-tillage (NT), reduced tillage (RT) and conventional tillage (CT), and three levels of fertilization (F1 - no fertilizer; F2 – 50 kg N/ha + 50 kg P/ha + 50 kg K/ha in fall, + 33.5 kg N/ha in spring; F3 - in 50 kg N/ha + 50 kg P/ha + 50 kg K/ha fall+ 93.5 kg N ha in spring), during 2014 at MRI Zemun Polje. The highest yields were observed with CT (12.19 t/ha), while the averages with RT and NT were lower (10.57 t/ha and 9.68 t/ha, respectively). The lowest yield was in F1 fertilization treatment 8.66 t/ha, while the yield was raised with amount of applied fertilizer (11.66 t/ha and 12.02 t/ha, respectively). The results of the influence of the amount of the applied fertilizers on protein content showed that the highest protein content have been obtained with treatment F2 (8.31%) followed by treatment F3 and F1. The average protein content at NT was 7.91%, while at RT, the protein content was 8.12% and at CT 8.17%. Also the highest oil content as well as starch content was with CT. The effect of fertilization on oil content is inconsistent, the highest content was in F2 treatment, 3,88%. The lowest protein, oil and starch content were in treatment without fertilizer. This experiment will continue.

Key words: grain quality, fertilizer, maize, tillage, yield

Introduction

Maize, the most important cereal crop of the world, is used for three main purposes as human food, feed for poultry and livestock, as well as for industrial processing. Maize grains have great nutritional value as they may contain 70-75% starch, 8-10% protein, and 4-5% oil. For successfully maize production optimal inputs are required. These include the use of adapted cultivars, appropriate tillage practice, application of fertilizers at amounts required for yielding potential, proper weed and disease control.

It is known that tillage affect soil properties, growth and yield parameters of maize. According to Khurshid et al., (2006) among the all crop production factors, tillage contributes up to about 20%. Tillage influences soil physical, chemical and biological properties, which in turn affect crop productivity (Anikwe and Ubochi, 2007). However, in Serbia is prevailing conventional or classical soil tillage system including considerable consumption of time, energy and human work.

Fertilizer incorporation is one of the most important farming operations needed to correct deficiencies in the soil in order to ensure proper growth and functioning of crops with the aim of increasing yield. Inadequate fertilizer rates lead to poor crop growth and reduced yields. On the other hand, over-fertilization leads to increased green biomass production and to lower grain yield and environmental pollution. Maize is particularly sensitive to soil nutrient deficiencies of both the major and minor nutrients and requires adequate supply of nutrients particularly nitrogen, phosphorus and potassium for good growth and high yield. Videnović et al (2011) studied effects of different tillage systems and level of fertilization on the yield of

the maize hybrid ZP 704 during ten years and find out the benefits of conventional tillage under the presented agro-ecological conditions, irrespective to the amount of applied fertilizer.

The chemical composition of maize grain is affected by many factors as fertilization, plant density, growing site etc. (Thomison et al., 2004). Higher protein content was obtained by conventional tillage in regard to no tilled soils (Andrija et al.,2009; Sessiz et al., 2010). According to Khan et al. (1992) grain protein, starch, and oil contents were not significantly affected by the different sowing methods. Javed et al. (1985) reported the stimulating effect of NPK fertilizers on protein content. Holou and Kindomihou (2014) studied the influence of fertilizer on protein, oil and starch in maize grain and find out that N fertilizers (up to 179 kg N/ha) increased protein and starch content in maize kernel.

The objectives of present study were to determine the impact of different tillage practices with different level of fertilizer on chemical composition and maize grain yield.

Material and methods

The field experiments arranged in a split plot design with three replicates were conducted during 2014 in Zemun Polje, in the vicinity of Belgrade in Serbia (44°52'N, 20°20'E), on a slightly calcareous chernozem. The maize hybrid ZPSC 606 was sown. The plant density was 59,524 plants/ha. The following tillage systems were investigated: NT – no-tillage, RT – reduced tillage and CT – conventional tillage. In the NT variant, sowing was performed directly with a drill. In the RT treatment, tillage was performed with a rotavator in the autumn to a depth of 10–12 cm and sowing was done with a conventional drill. The CT variant consisted of shallow plowing to a depth of 15 cm, immediately after wheat harvesting in 2013, primary tillage in the autumn to a depth of 25 cm, seedbed preparation in the spring with a Rau-combi (composed of a harrow, cultivator and rollers). The fertilizer treatments include: control F1 without fertilization; F2 – 50 kg N/ha + 50 kg P/ha + 50 kg K/ha in fall, + 33.5 kg N/ha in spring (up to 180 kg N/ha according to N-min); F3 - in 50 kg N/ha + 50 kg P/ha + 50 kg K/ha fall+ 93.5 kg N ha in spring (up to 240 kg N/ha according to N-min). The maize grain yield was measured at the end of the growing cycle from the two inner rows and calculated with 14% of moisture. The collected grain in harvest maturity was analyzed using Near Infrared Reflectance Grain Analyser (NIR), Chopin Technologies Infraneo. The seeds were analyzed for starch, protein and oil content. All observed data were analyzed using the analysis of variance (ANOVA). Treatment means were compared using the Fisher’s least significant difference (*LSD*) test ($P = 0.05$).

Results and discussion

A significant difference was observed in grain yield and seed chemical composition according to application of different tillage systems and level of fertilizers. The statistical analysis showed significant effects of tillage and amount of fertilizers on maize yield (Table 1). Soil tillage has also significant effect on starch content and no significant effect on protein and oil content (Table 2). Contrary, amount of applied fertilizers have significant effect on protein and oil content and non significant effect on starch content. Interaction between soil tillage and amount of fertilizers was significant for all traits.

Table 1. Influence of tillage systems and levels of applied fertilizers on grain yield

Treatment	T1	T2	T3	Average
F1	7,13a	8,36b	10,49c	8,66
F2	10,86d	11,45e	12,98g	11,77*
F3	11,02d	11,94f	13,09h	12,02*
Average	9,67	10,59*	12,19*	
	F	T	FxT	
LSD 0.05	0,155	0.164	0,241	

Table 2. Influence of tillage systems and levels of applied fertilizers on protein, oil and starch content

Protein content (%)				
	T1	T2	T3	average
F1	7,40a	7,85b	7,98c	7,74
F2	8,27f	8,33g	8,34g	8,31
F3	8,05d	8,16e	8,17e	8,13
average	7,91	8,12ns	8,16ns	
	F	T	FxT	
LSD 0.05	0.164	0.275	0,051	

Oil content (%)				
	T1	T2	T3	average
F1	3,74a	3,78b	3,82c	3,78
F2	3,86d	3,86d	3,92e	3,88*
F3	3,84c	3,86d	3,84c	3,85*
average	3,81	3,83ns	3,86ns	
	F	T	FxT	
LSD 0.05	0,043	0,057	0,038	

Starch content (%)				
	T1	T2	T3	average
F1	71,50a	72,50c	71,64a	71,88
F2	71,36a	72,87d	72,57c	72,27ns
F3	71,72b	72,54c	71,87b	72,04ns
average	71,53	72,64*	72,02*	
	F	T	FxT	
LSD 0.05	0,613	0,416	0,357	

Major differences between the tillage systems were noticed in F1 treatment. Results indicated the advantage of the CT system for grain yield (Table 1). The highest average yield was observed with CT 12.19 t/ha (100.00%), while the yield was lower with RT 10.59 t/ha (82.09%) and NT 9.67 t/ha (74.96%). Lower maize yields in no-till systems compared to conventional tillage systems are widely documented in other similar studies (Pederson and Lauer 2003, Boomsma *et al.* 2010, Videnovic *et al.*2011). Authors often report higher yields that can be achieved with conventional tillage in comparison to other non-conventional tillage systems (reduced, conservation and no-till). Borin and Sartori (1995), reported that among conventional tillage, minimum tillage and no-tillage in maize, the highest yield had been

obtained with the conventional tillage. Maurya (1988), also reported lower maize grain yield achieved with no-till system than with conventional tillage.
Yield reduction on the unfertilized plots compared to the F2 was the greater under conditions of no-till (37%) than on the reduced tillage (30%) and conventional tillage (20%). Averagely, the lowest yield was achieved with F1 treatment 8.66t/ha (100.00%), while the yields were higher in F2 (11,77 t/ha or 135,91%) and F3 treatments (12,02 t/ha or 138,79%). Kovačević et al. (2008) found out that different levels of P and K fertilization had a significant impact on maize yield. Similarly, lower yield was found in maize when the crop was subjected to the case of high N doses (Moser et al., 2006).
The effect of the interaction between the soil tillage and the amount of the fertilizers was significant at the RT and CT treatment for all fertilization levels. At the NT treatment a significant difference was observed between F1 and F2 and between F1 and F3, while there was no difference between F2 and F3. These results are in agreement with those reported by Shata et a*l*. (2007) and Sial et al. (2007). Adeniyan and Ojeniyi (2005) also harvested higher maize grain yield with the application of mineral and organic fertilizers.
The influence of tillage systems and level of fertilizers on chemical composition of maize seed was shown in Table 2. The results indicated that the effects of fertilizers level on the protein and oil content was significant. The protein content varied from 7,40% in NT-F1 treatment to 8,34% in CT-F2 treatment. Maximum protein content was recorded in CT system, as compared to RT and NT system, but differences between tillage systems are not significant. Comparing the protein content in regard to different fertilizer treatments (F1, F2, F3) for NT, RT and CT, the statistical differences were significant. Maximum protein content was obtained for F2 treatment in all three tillage variants. Application of nitrogen fertilizer had significant effect on maize seed protein content. Maize plots with fertilizer application had significantly ($P < 0.05$) higher protein content of 7,4% than maize plots without fertilizer application. According to Stone et al., (1998) and Hammad et al. (2011) low and high nitrogen dose have adverse effect on quality of maize. Khan et al (2008) reported higher N contents in the leaves being translocate and converted into proteins during kernel filling, suggesting that increased leaf area index, with increasing N availability, were the possible reasons for improved seed protein contents. The tillage system has no significant effect ($p \geq 0.05$), but fertilizer level showed significant ($p \geq 0.05$) effect on oil content. Oil content ranged between 3,74% to 3,92%. The average oil content was the highest in F2. Maximum oil content was in CT system with F2 level of fertilizer.
The tillage system had a significant ($p \geq 0.05$) effect, while fertilizers level had a no significant ($p \geq 0.05$) effect on starch content. In NT variant maximum starch content is obtained with F3 treatment, and in RT and CT with F2 treatment. In NT treatment average starch content was 71.55% followed with 72,02% in CT and 72,64% in RT. Maximum starch content has combination of RT variant and F2 level of fertilization. Maize grown without N fertilization promotes the greatest concentration of kernel starch, than kernels grown with the maximum N supply (Seebauer et al., 2010).

Conclusion

The combined effects of tillage systems with different level of fertilizers showed the significant effect at $p \leq 0.05$ for all traits. For the treatments F2 and F3 the differences were less expressed; hence, an appropriate application of fertilizers can reduce the differences appearing between the soil tillage systems. The treatment CT with F2 produced maximum protein and oil content followed by treatment RT+F2. Maximum yield was obtained with CT+F3 and starch content with CT+F2.The plants grown under CT treatment gave the highest grain yield, followed by RT treatment, while the plants grown under NT treatment gave the lowest grain yield.

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Review paper
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APPLICATION OF PLANT ANATOMY IN CROP RESEARCH

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Abstract

Anatomical aspects are an integral part of the resistance of plants to abiotic and biotic factors, influencing yield of cultivated plants. Plant anatomy is an important tool in agricultural research since understanding anatomical background of the mechanism of stress resistance is important for optimization of cultivation practices and for selection of new varieties in order to achieve higher productivity in stress conditions.

In this paper we present principals of techniques that are commonly used in plant anatomy research: brightfield light microscopy, fluorescence microscopy, polarization and electron microscopy, including some methods for chemical identification (histochemistry) and chemical imaging such as infrared and Raman microscopy with emphasis on some of their advantages and limitations and application in agricultural research. We here provided review on the methods most frequently used in crop plants investigations with emphasis on the research done at the Faculty of Agriculture University of Belgrade. We also present the methods currently used for imaging, measurements and image analysis using various software.

Keywords: *light microscopy, electron microscopy, fluorescence microscopy, chemical imaging, image analysis*

Introduction

Abiotic factors have a significant impact on the growth and development of cultivated plants and can influence to a large extent their yield. Drought is considered as one of the most common abiotic stress factors, and the following properties of leaves are referred as the adaptation of plants to water deficit: greater number of stomata per unit area, smaller epidermal cells, thicker cuticle, smaller mesophyll cells, compact leaf mesophyll, more layers of cells in the leaf mesophyll, thicker leaves, size and functionality of vascular tissues, etc. (De Micco and Aronne, 2012, Quarrie et al. 2006).

Anatomical studies may provide an answer to questions concerning the influence of biotic factors on plants, nature and intensity of the damage caused by the activity of herbivorous organisms (insects, nematodes ...), pathogens (bacteria, fungi, viruses ...) or parasitic plants, and possible non-specific and specific defense reaction of plants on these organisms (De Cal et al. 2000, Rančić et al. 2006, Pećinar et al. 2011). Anatomical study of the roots of plants, especially in cases where there are symbiotic relations (mycorrhiza and nitrogen fixation) are important for understanding the adoption of water mineral substances (Dreyer et al. 2010). Studying the flower nectaries can provide the basis for a better understanding of possible mechanisms of nectar secretion and different aspects of attacking pollinators (Nedić et al. 2013, Mačukanović-Jocić et al. 2007). In addition, studies of viability and germination of pollen and cytological and histological study of fruit development (Rančić et al. 2010a, Pecinar et al. 2013) are directly related to the potential increase in yields of cultivated plants. Also, anatomical investigations could have scientific value in terms of our general understanding of the parasite-host interaction (Rančić and Božić, 2004), as well as a practical one by providing data that are essential for a more efficient application of adequate control. In addition, for agriculture are also important anatomical characteristics of weed species

(Vrbničanin et al. 2007, Božić et al. 2008). Anatomical studies are important in studying the mechanisms of action of certain herbicides. Knowledge of population variability in the anatomy of weed species, as well as differences between individuals of the same species may be important for herbicide resistance and thus affect their control. Specific morphological features of plants, such as cuticle on the leaves, the presence of different types of hair, number of stomata per unit leaf area, affect the adoption of herbicide by leaf surface (Solymosi and Nagi, 1998; Grangeot et al.. 2006), and thus the expression of their efficiency. In *Cirsium arvense* (L.) Scop. it was shown that the various varieties differ in sensitivity or resistance to the herbicide (Hodgson, 1970; Zand i sar., 2002; Vrbničanin i sar., 2004).

Microscopy techniques

Microscopy techniques are usualy divided into three well-known branches*:* optical, electron, and scanning probe microscopy. The techniques that are commonly used in anatomical studies of plants are optical and electron microscopy, although increasing importance of techniques that allows microanalysis and which in addition to information about the structure provide information on the chemical composition. Methods involving the use of nanomikroscopes (scanning probe microscopy) are still not used a lot in plant anatomy.

Optical microscopy

The first invented and still the most common type of microscope in biological speciment research is optical microscope, referred also as light microscope. It uses visible light and a system of lenses to magnify images of small samples and could be based on transmitted or reflected light.

In transmitted light microscope (compound microscope) sample is illuminated from the side that is opposite to the lens, and sample should be transparent enough to enable light to pass the observer's eye. That is the reason why samples are usually cut on very thin sections before observations. Sometimes it is possible to observe the whole plant organ but only if it is transparent enough to allow passing of the light, therefore an alternative to the cutting can be tissue clearing that is most commonly used in studying intact structures in flowers. The transmitted light microscope is used for observation of samples whose size is from a few micrometers to a few millimeters. This microscope is useful for observing plant internal structures, different cell types, some organelles (plastids, nucleus and vacuoles) and metabolic products such as crystals of calcium oxalate or starch. As this type of microscope has a very low contrast, most of the samples require additional staining. Maximal resolution in light microscopy is approximately 0.2 micrometers, what limits the practical magnification is up to 1500 times. This type of microscopy is the most widely used in the investigations of the crop vegetative organs such as stem (Fig. 1a) and leaf (Fig. 1b).

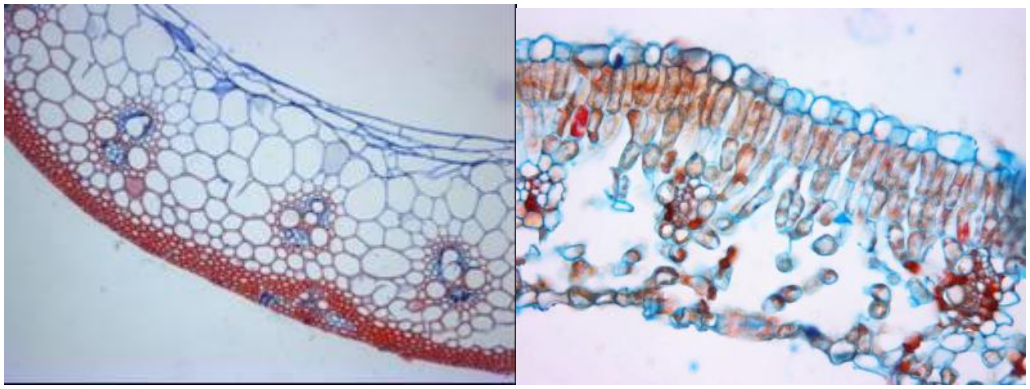

Figure 1. Transversal section stained with safranin and alcian blue: a) wheat stem and b) apple leaf

In reflected light microscope (stereomicroscope) sample is usually illuminated from the same side as the lens, so the sample does not need to be transparent. It is a lower powered microscope comparing to transmitted light microscope, but it provides a stereoscopic view of the sample and is valuable in situations where three-dimensional observation is required. Stereomicroscopes is useful for observation of larger samples (up to a few centimeters) and when micromanipulation of the specimen is required. In some circumstances, reflected and transmitted light sources could be combined to take advantage of particular specimen characteristics in a manner that most effectively reveals the features of interest.

Darkfield microscopy

Darkfield microscope is transmited or reflected light microscope which has a filter enabling illuminating the sample with light that will not be collected by the objective lens, what produce black background with bright objects on it. Darkfield microscopy is used to enhance the contrast of unstained samples. and is especially useful for observing living cells or for finding cells or organels (chloroplasts, mitochondria) in suspension. The tissue needs to be strongly illuminated, which may damage delicate samples

Polarizing Microscopy

Polarized light microscopy is optical microscopy which include illumination of the sample with polarized light to investigate its optical properties. Polarized light microscopy can be used both with reflected and transmitted light. Polarization provides information on absorption, structure and composition of the samples and this type of microscopy is suitable for study of crystalline and fibrous structures, which rotate part of polarized light and thus become more visible. The polarizing microscope could be applied to observe section of fresh, unfixed materials, and it is especially suitable for the study of starch grains of different plants species (Fig. 2).

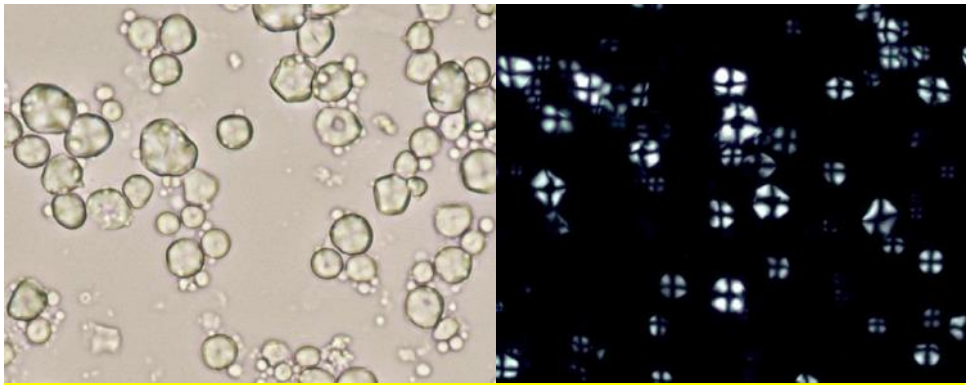

Figure 2. Corn starch grains observed in the bright field microscopy (a) and polarized light microscopy (b)

Phase contrast microscopy and Differential interference contrast (DIC) microscopy

Phase contrast is an optical microscopy illumination technique that shows differences in refractive index as difference in contrast. Due to improving image contrast it reveals many cellular structures that are not visible with a simpler bright field microscope, so it could be used for observing unstained, transparent samples. Similar to Phase contrast microscopy is

Differential interference contrast microscopy also known as Nomarski Interference Contrast (NIC) or Nomarski microscopy which requires a polarized light source to function. DIC is capable to enchance contrast even more, but is unsuitable for thick samples, such as tissue slices and highly pigmented cells.

Fluorescence microscopy

Fluorescence is an optical phenomenon in which light from shorter wavelength is absorbed by the substance and then emitted as longer wavelengths light. Fluorescence microscope is designed for observation of the samples which fluoresce in its natural form (primary fluorescence or autofluorescence) or fluoresce when treating some fluorescent chemicals (secondary fluorescence). A fluorescence microscope works on the same principle as an ordinary light microscope, but as a source of short wavelength of light used mercury or xenon lamp. Fluorescence microscopy is a powerful tool for the study of plant structure. The main value of this technique is the ability to detect substances that are present in very low concentrations. Using the epi-fluorescence it is possible to observed surface of the samples that are relatively large and it is not necessary to be transparent. Many compounds in plants such as chlorophyll, lignin, suberin and phenolic compounds have the ability to exhibit autofluorescence, so it is possible to identify some of these substances very easily, even on hand sections without staining (Fig. 3). Samples which do not have the property of autofluorescence should be pretreated with special fluorescent dyes (fluorochromes). Many specific fluorescent probes have been developed to allow observing large number of different macromolecules, such as callose in phloem or nucleic acid.

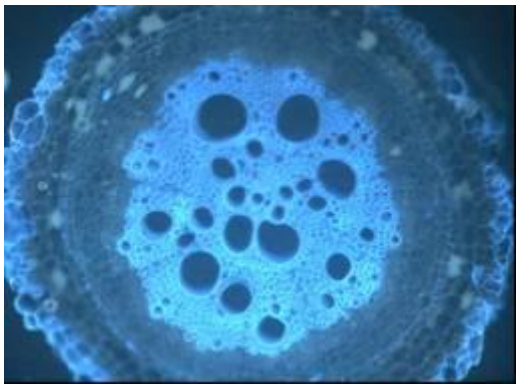

Figure 3. Cross-section of tomato root in epifluorescence microscope (blue- autofluorescence of lignin)

Fluorescent dyes are especially suitable for vital staining, because the very small amount of the fluorescent substance can be detected by fluorescence microscopy. In the indirect technique fluorochromes are used first to bind to the protein or some other molecule, and after that protein carrying fluorescent dye binding to the receptor site in the tissue sample. This reaction is the basis of the immunofluorescence. Developing of fluorescent probes, as well as improvements in optical microscopes and light sources (laser), as well as computers and appropriate software, enabled design of confocal microscopes, special type of fluorescence microscope which enabling optical sectioning of the sample and subsequently 3D reconstruction of obtained pictures.

Electron Microscopy

The electron microscope uses a beam of electrons instead light to create an image of the sample. The most important variants of electron microscopy are transmission electron microscopy and scanning electron microscopy.

In Transmission Electron Microscope (TEM) the electrons in the primary beam are transmitted through the sample. TEM has a very large magnification range (up to 50 million times) and the ability to obtain images of very high resolution, better than 50 pm.. In plant anatomy research, TEM is mainly used for analyzing the internal structure of the cells - cell organelles. Samples prepared for electron microscopy must be very thin, about 100 nm or less. Electron microscopy is not capable for observing living cell because all life processes in the sample must be stopped by quick freezing or chemical fixation. Sample preparation in addition to fixation involves chemical dehydration too and embedding in the plastic or resin that allows cutting of very thin (ultrathin) section. Cutting embedded material requires specific microtome called ultramicrotome, which instead of a metal blade have glass or diamond knives. Visualization and contrasting preparation for electron microscopy is achieved by the addition of heavy metal salts, which bind to cellular components, what manifested as different in their densities. Transmission electron microscopes produce two-dimensional, black and white images (Fig. 4), but by combining a large number of 2D images in softvares is possible to get a 3D view. Proteins or other molecules can be located by applying antibodies conjugated to gold particles.

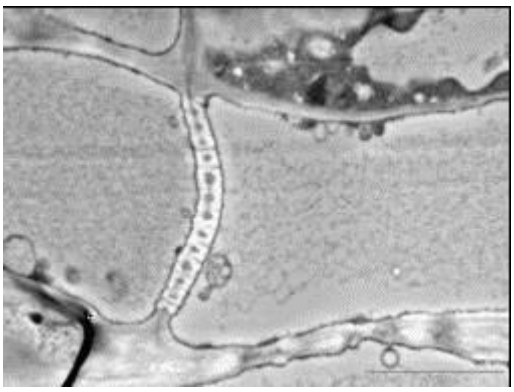

Figure 4. Longitudinal section of sieve tube and sieve plate in tomato pedicel observed in TEM

In Scanning Electron Microscope (SEM) images are produces by detecting secondary electrons which are emitted from the surface due to excitation by the primary electron beam. Comparing to TEM which have much better resolution and magniffication (up to about 500.000 times), SEM has a much greater depth of view, provide us with 3D, topographical information about the sample surface (Fig. 5). Preparing samples for SEM is easier than for TEM and since the sample could be up to a few centimeters sized, thin sectioning is not necessary. Most biological samples need to be dehydrated and coated in a conductive material before being placed in the SEM. If combined with x-ray microscopy, beside topography information, SEM could be capable of to provide precise information of the chemical composition of the material near its surface (elemental analysis).

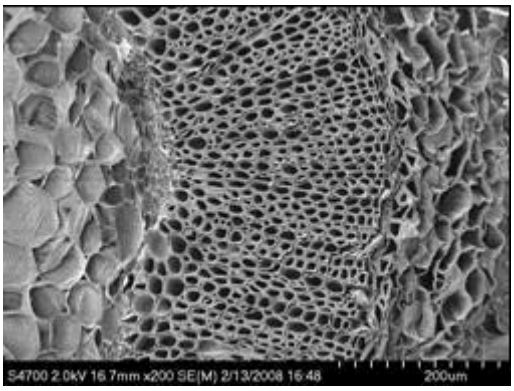

Figure 5. Tomato pedicel transfersal section observed in SEM

Scanning probe microscopy

Scanning probe microscopes are very powerful microscopes which could be used to investigate surfaces with atomic resolution. There are several types of Scanning probe microscopes: atomic force microscopes, scanning tunneling microscopes etc. and they use very sharp probe to scan the surface of a sample. The probe in these microscopes does not touch the surface but traces the specimen nanometers above the surface. During this scanning process, a computer gathers data of the interaction of a scanning probe with the surface of the sample to generate an image. In addition to visualizing nanoscale structures, some of these microscopes can be used even to manipulate individual atoms. Although there are some examples of using nanomicroscopes in biological research, e.g. for the investigating surface of a living cell, these microscopes have much more used in material science research.

Selected anatomical methods and examples of their application in agricultural research

Methods for studying epidermis

The surface of the leaves and other plant organs can be observed directly by stereomicroscope - light microscope using reflected light, or by fluorescence microscope or scanning electron microscope. For transmitted light microscopy slides are usually prepared by the method which includes peeling off the epidermis (Weyers and Travis, 1981) or making imprint by applying clear nail polish (Fig. 6) (Miller-Rushing et al. 2009). This methods enabling studying epidermal cell shape, size as well as abundance of atypical epidermal cells: glandular and nonglandular trichomes, stomata etc.

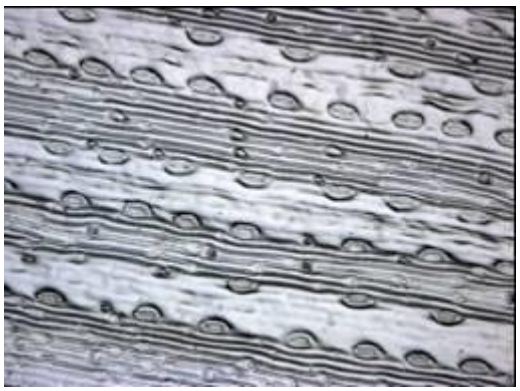

Figure 6. Nail polish imprint of leaf surface of wheat

Methods for studying hydraulic network

Apoplastic water flow through the plant can be traced by infiltration of different dyes dissolved in water. The dye could be infiltrated into xylem tissue by immersing freshly cut basal part of the stem into the staining solution. After some time, due to transpiration, dye will be sucked into xylem vessels. Since dye solution passes only through functional xylem vessels, comparing the area of stained and non-stained vessels it is possible to get information of the ratio of functional and non-functional xylem elements (Rancic et al. 2010b) (Fig. 7).

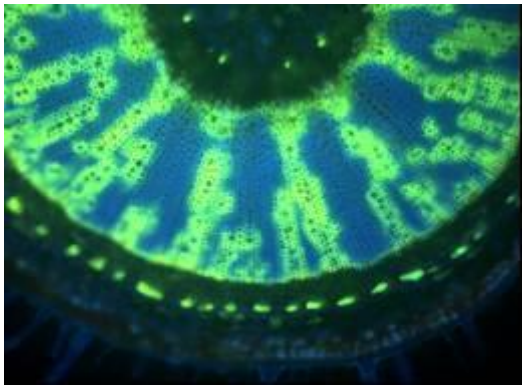

Figure 7. Functional analysis of xylem in tomato pedicel: blue-xylem autofluorescence; yellow - functional xylem

Simplastic water transport through the plant is possible to visualize using an aqueous solution of colors which transported by phloem over long distances, and which due to the characteristic of the cell membrane does not diffuse into the surrounding tissue (Rančić et al. 2011). Such dyes are usually added over the leaves, and through the stomata penetrate into the leaf parenchyma, and later were transported by phloem to other parts of the plant.

Methods for the investigation fruit anatomy and fruit cytology

Anatomical analyses of fruits could be used for monitoring development of certain plant organs. Histological researches performed by tissue sectioning (Fig. 8) or cytological analysis after tissue maceration (Fig. 9) could be used. Histological sections enabling observing different tissue layers in plant organ, while method of tissue maceration enabling dissolving median lamella connecting the adjacent cells and separating from each other, thus allowing us to study the three-dimensional aspects of the of cells. Mmethod of maceration could be used for investigation all tissue types, but the most offen is used for vascular tissue and fruit investigations (Pećinar et al. 2014).

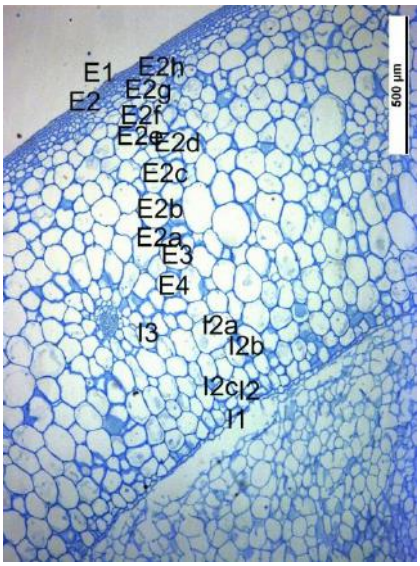

Figure 8. Transfersal section of tomato fruit pericarp

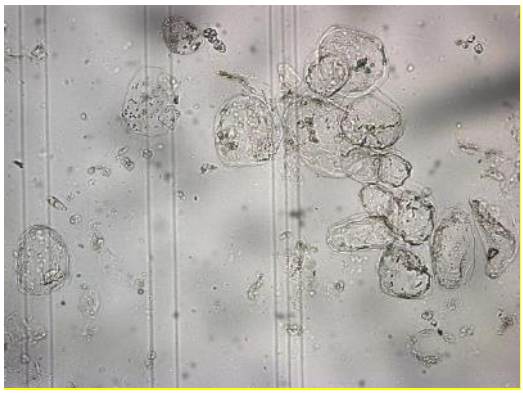

Figure 9. Macerated tissue of tomato fruit pericarp

Identification and localisation of different plant metabolites

Histochemical staining

Anatomical studies can also be used to assess the quality of the product. This could be achieved by application of histochemical staining, specific test for some group of compounds allowing to selected cell structures to be differentiated. These tests may indicate the chemical nature of the colored substance, and are often used for the identification and localization of various reserve materials (starch, proteins, lipids) (Fig. 10) or secondary metabolic products that play a role in the plant defense (different phenolic compounds, tannins, alkaloids, flavonoids) (Marin et al. 2006, Rančić et al. 2009, Rancic et al. 2013, Pljevljakusic et al. 2012).

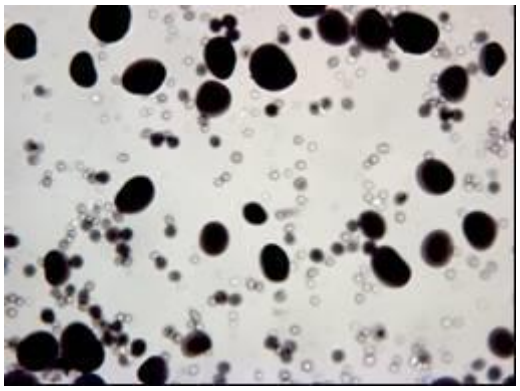

Figure 10. Wheat starch grains stained black by Lugol reagens

Microscopes for microanalysis

More recently are developed microscopes that enable not only a visualization of the sample but also chemical analysis of the sample (microscope for microanalysis). With these microscopes is possible to localize different molecules or groups of compounds in the sample and the technique is named chemical imaging. A special advantage of this method is that it is non-invasive and usually does not require staining or specific sample preparation.

Infrared microscopy

Infrared microscope is configuration of an optical microscope, a infrared spectroscope (usually Fourier Transform Infrared Spectrometer - FTIR) combined with and an infrared detector. Infrared spectroscopy study infrared radiation emitted by the molecules due to their vibration. Free atoms do not emit infrared radiation, but molecules after absorption infrared radiation begin to vibrate, and each molecule has a characteristic vibration, ie has a different infrared spectrum which can be used for the identification of the specific compounds. FTIR microscope is not suitable for observing water solutions and fresh plant samples, due to strong signal of water molecules vibration, but it is useful for dry samples (i.e. seeds). In infrared microscopes the spatial resolution is relatively low, limited to 1-3X the wavelength, what sets a practical spatial resolution limit of ~3-30 μm.

Raman microscopy

Raman microscope is very specialized optical microscope in which the sample is illuminated by monochrome light (the light source is a laser) and light scattered by the observed material is analyzed by Raman spectrometer. Analysis of scattered light could give information about the chemical composition of the material and could be analyzed at different points in the sample giving chemical image of the sample. Applications of this technique in the field of plant science are very far ranging, going from investigations on structural polymers to metabolites and mineral substances (Gierlinger and Schwanninger 2007, Agarwal, 2014). Non-destructive nature of Raman analysis along with none-to-minimal requirement of sample preparation makes it exceptionally useful for various investigations (i.e. cellulose and pectin: Atalla and Agarwal 1986; Gierlinger et al. 2010, carotenoids in tomato fruit: Qin et al. 2012; Baranska et al. 2006, starch, lipid and proteins in wheat grain Manfait et al. 2004) (Fig. 11). The informations provided by Raman are complementary to information obtained by infrared (IR) (Lang et al. 1986). In contrast to FTIR, Raman is useful for styding fresh plant samles rich in water and have better resolution (0.5-2 micrometers) but the main problems associated with the use of conventional Raman on plant materials is strong autofluorescence that is produced when phenolic compounds (i.e., lignin) are excited by visible light. This problem

could be overcome by using longer wavelenght laser source or NIR-FT-Raman (Agarwal, 2014).

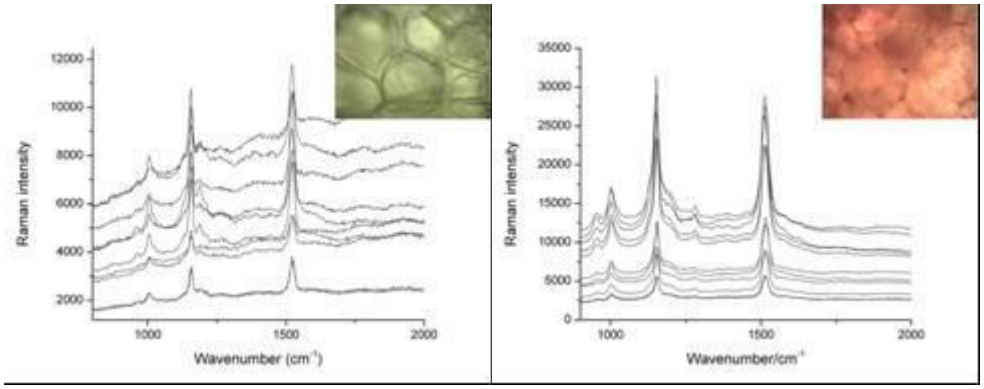

Figure 11. Raman spectra of tomato fruit obtained at 532-nm laser at two developmental stages. Figure 11a indicates on mature green, and Figure 11b on over ripe fruit stage.

Raman peaks appeared consistently at 1001 and 1151 cm^{-1} (indicating the position of C-C bond) in mature green fruit stage and ripe fruit. The third peak was gradually shifted from 1525 cm^{-1} (lutein at mature green stage) to 1513 cm^{-1} (lycopene at red stage), indicating the position of C=C stretching vibration of the polyene chain in carotenoids, owing to the loss of lutein and β-carotene and the accumulation of lycopene during tomato ripening.

Acquiring and measuring of microscopic preparations

At the same time with development of microscopy, different methods for measurement of microscopic objects have been developed. The simplest technique involved the direct comparison of the size of the object with a graduated scale in eyepiece, however the latest digital camera connected to the microscope, combined with powerful computer software, enables quick and easily performed microscopic measurement and automatic data analysis. Analysis of the picture varies widely depending on the type of the sample and research needs. Typical applications for automatic image analysis involves determining the edges of the objects (e.g. cell wall), counting the number of similar objects (e.g. nuclei), the measurement of area, length, volume and other characteristics (Fig. 12). Software for analysis of allows users to manipulate images in many different ways, such as creating 3D images, making video sequences that follow the movement of certain objects or its parts cells, etc. Progress in programming led to the development of software that enabled the fully automated operation of the microscope, which saves time and increases the quality of the research. In addition to commercial programs designed by the with eminent companies for the production and distribution of microscopes and related equipment (eg Nikon, Zeiss, Leica, Olympus ...), online could be find free programs for image analysis too, such as ImageJ (http: //imagej.nih.gov/ij/), Fiji (http://fiji.sc/), Icy (http://icy.bioimageanalysis.org/), Cell Profiler (http://www.cellprofiler.org /) and others. For the processing of spectra obtained by Raman or FTIR microscopy are commonly used Matlab and R softwere, while for primary processing of the spectra without additional statistical tests Origine, PeakFit and LabSpec softweres could be enough.

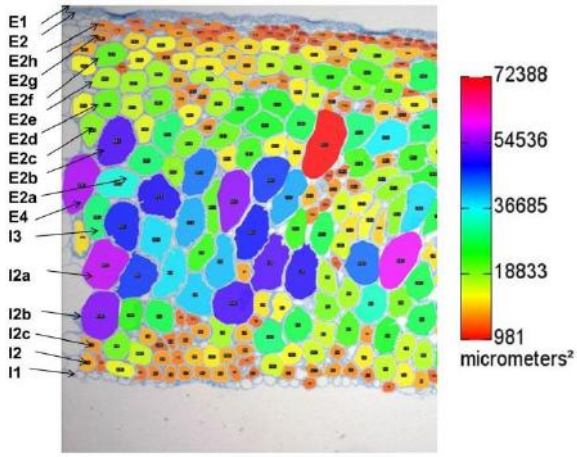

Figure 12. Tomato fruit pericarp cells automatically colored according to their size by the ROI color coder plugin of Image J software.

Acknowlegdement

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GENETIC POTENTIAL AND YIELD COMPONENTS OF WINTER BARLEY

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Abstract

The interaction of genotype x environment on yield and 1000 grain weight and test weight at four KG cultivars of barley (Rekord, Jagodinac, Maksa and Grand) was researched in this paper. The experiment was established on the experimental field of the Small Grains Research Centre in Kragujevac (Serbia) during two growing seasons. The highest grains yield per area unit had, on an average, Jagodinac and Grand cultivars (6.647 t/ha and 5.919 t/ha). Average grains yield observed in the two-year period was the highest at Jagodinac cultivar (6.647 t/ha), while the lowest yield was obtained by Grand and Maksa cultivars (5.919 t/ha and 5.742 t/ha). The largest two-year average value of test weight was found in the cultivar Maksa (69.56 kg/hl), and lowest in cultivar Grand (64.87 kg/hl). During the both years of investigation the highest average value of 1000 grain weight achieved the Maksa cultivar (49.84 g and 47.85 g). The highest average 1000 grain weight was determined for cultivar Maksa (48.84 g). Very highly significant differences in 1000-grain weight and highly significant of grain yield and test weight at investigated barley were found in relation with the cultivar. During 2006-2007, statistically significantly higher grain yield per area unit as well as 1000 grain weight was achieved, compared with 2005-2006. Interaction of the year and cultivars on the yield and 1000 grain weight were statistically significantly. Investigations on genotype and environment interaction present the basis for further refinement and barley zoning.

Key words: *cultivar, grain yield, winter barley*

Introduction

Barley cultivars that were in production until the end of the eighties were characterized by the lower yields, good technological quality and higher stem sensitive on lodging. Due to lower resistance on lodging, that cultivars were grown at modest soils and therefore they had lower yields (Đekić et al., 2012.b). Agronomic cultivar value depends not only on its genetic potential for yield, but also on its ability to achieve genetic potential under different conditions of production (Mladenović et al., 2009). New varieties are characterized by good technological quality, better resistance on lodging and diseases, shorter stem and more efficient assimilates usage (Pržulj et al., 2004). Malting barley is expected to have 1000 grain weight of 40 and 46 g and test weight of 68 to 75 kg/hl (Paunovic et al., 2006). Yield is largely dependent on the genetic potential, which could be defined as yield of variety which was grown in conditions on which it had been adapted, with adequately amounts of water and nutrients and efficient control of pests, diseases, weeds and other stresses (Đekić et al., 2011). Yields considerably vary primarily as a result of agro-ecological conditions during the growing season (Đekić et al., 2014).

Barley is in the usage as an important component of beer and malt production, important and quality component in animal feeding, partially in human nutrition as well as a component in processing industry (Đekić et al., 2012.a). As a top-quality forage crop plant, barley is used as fodder unit in meals balancing in animal feed (Đekić et al., 2010).

The aim of this study was determination of the cultivars and ecological environmental factors influence on differences in stability and adaptability of cultivars regard the grain yield, 1000 grains weight and test weight of tested winter barley cultivars, as well as specificity cultivars exploring regard growing seasons conditions.

Materials and methods

Experimental design

During the 2005-2006 and 2006-2007 growing seasons, four cultivars of winter barley (Rekord, Jagodinac, Maksa and Grand), cultivated at the Center for Small Grains in Kragujevac (Serbia) were investigated. Experiments have been conducted in randomized block systems, with a plot size of 5 m^2 (2 m x 2.5 m) in five replicates. The usual techniques for barley production were applied, and it was done in the optimum sowing time in late October. 400 kg/ha of fertilizer NPK 15:15:15 was added in the fall on the investigated plots, while during the spring fertilization, 300 kg/ha (KAN) was supplemented. The following properties were analyzed: grain yield (t/ha), 1000 grain weight (g) and test weight (kg/hl).

Meteorological conditions

Kragujevac area is characterized by a moderate continental climate, which general feature is uneven distribution of rainfall by months. Data in Table 1 for the investigated period (2005-2007) clearly indicate that the years in which the researches were conducted differed from the typical multi-year average for Kragujevac region, regarding the meteorological conditions.

Table 1. Mean monthly air temperature and precipitation (Kragujevac)

Months	Mean monthly air temperature (°C)			The amount of rainfall (mm)		
	2005-06	2006-07	Average	2005-06	2006-07	Average
VIII	20.0	20.7	22.7	117.8	141.9	58.5
IX	17.4	17.7	16.6	115.6	57.4	62.7
X	11.5	13.3	12.5	49.0	16.7	45.4
XI	5.7	7.6	6.9	54.8	13.7	48.9
XII	3.4	3.5	1.9	47.9	51.9	56.6
I	-1.7	6.1	0.5	27.9	45.3	58.2
II	1.5	6.3	2.4	38.1	32.1	46.6
III	5.6	9.1	7.1	116.1	62.9	32.4
IV	12.7	12.1	11.6	86.3	3.6	51.9
V	16.4	18.2	16.9	29.6	118.4	57.6
VI	19.7	22.8	20.0	84.8	25.3	70.4
VII	23.0	24.8	22.0	22.4	10.1	71.5
Average	11.27	13.52	11.76	790.3	579.3	660.7

The average air temperature in 2005-2006 was lower by 0.49°C and 2006-2007 was higher by 1.76°C. The sum of rainfall precipitation in 2005-2006 was higher by 129.6 mm, where the sum of rainfall in 2006-2007 was 81.4 mm lower than the average of many years and with a very uneven distribution of precipitation per months. Spring months March and April in 2005-2006 were surplus of precipitation, what affected unfavorable on the crops. During the March in 2005-2006 it was 116.1 mm of rainfall, what was 83.7 mm more compared with the perennial average. Regard the high importance of sufficient rainfall amounts during the spring months, particularly May for small grains production, the distribution and amount of rainfall

over the growing season 2006-2007 were considerably more favorable, what resulted with increment of yields during that year. Apart from the rainfall deficiency during the spring months and the non-uniform distribution of rainfall across months, an increasing in average air temperatures was also observed.
Based on the fact that sufficient amounts of rainfall in these months are very important for the successful production of cereal crops it can be concluded that the years in which the researches were conducted were not favorable for the barley growing.

Soil and weather conditions

Before the commencement of the experiment soil samples were taken from the sample surface and the chemical analysis of soil was performed. On the basis of obtained results it was revealed that the soil belongs to the smonitza type, with relatively high clay content, and unfavorable physical properties. The humus content in the surface layer of soil was low (2.38-2.64%), and a substitution and total hydrolytic acidity were quite high (pH H_2O=5.99, KCl=4.56). The soil was medium provided with total nitrogen (0,11-0,13% N) and easily accessible potassium (10-14 mg/100 g soil K_2O), while the available phosphorus content was low (under 10 mg/100 g of soil P_2O_5).

Statistical Analysis

On the basis of achieved research results the usual variational statistical indicators were calculated: average values, standard error and standard deviation. Statistical analysis was made in the module Analyst Program SAS/STAT (SAS Institute, 2000).

Results and discussion

Average values of yield (t/ha), test weight (kg/hl) and 1000 grain weight (g) at investigated Kragujevac's winter barley cultivars grown at the Center for Small Grains in Kragujevac during two growing seasons, 2005-2006 and 2006-2007, are presented in the Table 2.

Table 2 Average values of investigated barley cultivars characteristics

Traits	Cultivar	2005-2006			2006-2007			Average		
		x	S	Sx	x	S	Sx	x	S	Sx
Grain yield (t/ha)	Rekord	4.940	0.356	0.159	5.272	0.669	0.299	5.106	0.535	0.169
	Jagodinac	6.242	0.443	0.198	7.052	0.557	0.249	6.647	0.638	0.202
	Maksa	5.184	0.695	0.311	6.300	0.503	0.225	5.742	0.820	0.259
	Grand	4.770	0.488	0.218	7.068	0.463	0.207	5.919	1.292	0.408
Test weight (kg/hl)	Rekord	66.30	2.086	0.933	63.79	3.490	1.561	65.04	3.016	0.954
	Jagodinac	70.81	2.627	1.175	66.37	5.050	2.259	68.59	4.459	1.410
	Maksa	70.35	1.038	0.464	68.77	1.178	0.527	69.56	1.337	0.423
	Grand	63.57	2.906	1.300	66.18	1.227	0.549	64.87	2.513	0.795
1000 grain weight (g)	Rekord	49.76	2.431	1.087	43.76	1.846	0.826	46.76	3.760	1.189
	Jagodinac	44.36	0.684	0.306	42.61	0.872	0.390	43.48	1.182	0.374
	Maksa	49.84	1.799	0.805	47.85	1.966	0.879	48.84	2.063	0.652
	Grand	39.16	0.865	0.387	37.56	1.545	0.691	38.36	1.451	0.459

During the first year of investigations, cultivar Jagodinac achieved the highest grains yield (6.242 t/ha), followed by Maksa (5.184 t/ha), while the lowest yield was at Grand cultivar (4.770 t/ha). During the second year of investigations (2006-2007), the yield of Grand cultivar was the highest with 7.068 t/ha, while the slightly lower yield was realized by Jagodinac

cultivar (7.052 t/ha). Average grains yield observed in the two-year period was the highest at Jagodinac variety (6.647 t/ha), while the lowest yield was obtained by Grand and Maksa cultivars (5.919 t/ha and 5.742 t/ha). Considerable variation in yield depending on years of research have established Jelić et al. (2007), Đekić et al. (2011) and Popović et al. (2011). Grain yield is a value, which is not only genetically determined but also dependent to a high degree on the growing conditions (Popović et al., 2011, Sabaghnia at al., 2013, Chamurliyski et al., 2015).

During the first year of investigations, cultivar Jagodinac achieved the highest test weight (70.81 kg/hl). The barley cultivar Maksa has achieved the highest test weight in both years of investigation compared to other tested barley cultivars (70.35 kg/hl and 68.77 kg/hl). The average two-year value of test weight at Maksa cultivar was 69.56 kg/hl, Jagodinac 68.59 kg/hl, Rekord 65.04 kg/hl, while the lowest average was at Grand cultivar (64.87 kg/hl). Grain of investigated barley cultivars was characterized by good physical characteristics; especially regard the test weight and 1000 grain weight. Realized average values of these characteristics in the study were slightly higher than the values obtained by Jelić et al. (2007) and Đekić et al. (2011).

During the both years of investigation (2005-2006 and 2006-2007), Maksa cultivar achieved the highest average 1000 grain weight (49.84 g and 47.85 g) compared with other tested barley cultivars. During the first and second years of investigation the lowest average value of 1000 grain weight achieved the Grand cultivar (39.16 g and 37.56 g).

Table 3 Analysis of variance of the tested parameters (ANOVA)

Effect of year on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 1, 38	p-level
Grain yield (t ha^{-1})	12.97321	0.69462	18.67660***	0.000108
Test weight (kg hl^{-1})	21.90400	12.78997	1.71259	0.198509
1000-grain weight (g)	80.37225	19.55583	4.10989*	0.049690
Effect of cultivar on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 3, 36	p-level
Grain yield (t ha^{-1})	4.0171	0.758825	5.29380**	0.003973
Test weight (kg hl^{-1})	58.0604	9.270597	6.26286**	0.001563
1000-grain weight (g)	208.8027	5.474597	38.14029***	0.000000
Effect of the year x cultivar interaction				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 3, 32	p-level
Grain yield (t ha^{-1})	1.75275	0.283945	6.172851**	0.001970
Test weight (kg hl^{-1})	22.13383	7.669875	2.885814	0.050828
1000-grain weight (g)	11.19475	2.597781	4.309350*	0.011612

**Statisticaly significant difference (P<0.05) **Statisticaly high significant difference (P<0.01)*

Table 3 shows the impact of year, cultivar and interaction of year x cultivar on yield, test weight and 1000-grain weight. The ANOVA indicated very highly significant effects of the year and grain yield (F_{exp}= 18.6766***) and significant effects of the 1000 grain weight (F_{exp}=4.10989**). Our results are consistent with the results Madić et al. (2009), where the authors state that the growing conditions in the observed years had a significant impact on yield. Very highly significant differences in 1000-grain weight and highly significant of grain yield and test weight at investigated barley were found in relation with the cultivar. Furthermore, 1000-grain weight was significantly affected by the year x cultivar interaction

($p<0.05$). The interaction of grain yield and year x cultivar very significant differences at investigated barley cultivars ($p<0.01$).

Achieved statistically significantly higher yields in 2006-2007 were, primarily, the result of heavy rainfalls and their good distribution as well as favorable air temperatures during the vegetation period (Table 1). Đekić et al. (2014) in his research states that the air temperatures and the rainfall amount and distribution during the barley growing season have the greatest impact on high yields and grain quality.

Conclusion

Based on the gain results during two-year investigation on four Kragujevac's winter barley cultivars, it can be concluded that the highest yield achieved the cultivar Jagodinac (6.647 t/ha). Maksa and Grand cultivars have achieved satisfactory results, while the poorest results were achieved by the cultivar Rekord. During 2006-2007, statistically significantly higher grain yield per area unit as well as 1000 grain weight was achieved, compared with 2005-2006. Significant differences in 1000 grain weight at investigated barley cultivars were found relative to the cultivar and very significant differences at grain yield relative to environmental factors. Very highly significant influence of the cultivar on 1000 grain weight was established at investigated winter barley cultivars by variance analysis, while genotype influence on grain yields and test weight was highly statistically significant.

Grain quality is a qualitative characteristic affected by genetic and environmental factors, as well as their interactions, and therefore the cultivar in individual years can acts as a beer or forage cultivar, what can be test determined and that way the grain can be direct for a particular purpose.

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EFFECTS OF FERTILIZATION ON YIELD AND GRAIN QUALITY IN WINTER WHEAT

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Abstract

The study was carried out in a stationary field trial involving fertilization over a two years period from 2008/09 to 2009/10. Trials were first set up in the experimental fields of the Small Grains Research Centre in Kragujevac in 1970. The wheat cultivar used in the experiment was Vizija, the dominant cultivar in the production region of Serbia. Studies of fertilization effects were conducted in a stationary field trial on a degrading vertisol soil with low pH. Eight variants of mineral nutrition (control, NK_1, NP_1, NP_2, NP_3, NP_1K_1, and NP_2K_1) and untreated control (without nutrition) were tested in the experiment. The highest grain yields under mineral nutrition involving a combination of three mineral elements NP_1K_1 (4.827 t/ha) and two mineral elements NP_3 treatment (4.564 t/ha). Over the two-year period, all investigated treatments of wheat achieved the highest average test weight in the NP_2 (76.545 kg/hl).

Based on the analysis of variance, it can be concluded that two are highly significant differences in grain yield regard the year of investigation ($F_{exp}=12.033^{**}$) and significant differences at 1000-grain weight ($F_{exp}=8.216^{*}$) and highly significant differences in test weight ($F_{exp}=121.874^{**}$). Highly significant differences in grain yield at investigated wheat cultivar were found relative to the fertilization. In the dual interaction between the year and fertilization, significant effects were highly significant on grain yield and 1000-grain weight.

Grain yield were significantly positively correlated with 1000-grain weight only in the 2009/10 ($r=0.45^{*}$). Test weight in 2008/09 was positively correlated with 1000-grain weight ($r=0.47^{*}$).

Key words: *fertilization, yield, quality, wheat*

Introduction

New perspective wheat lines and varieties has more and better filled grain, higher yield, grain mass and farinaceous content, while proteins and lysine was smaller compared with older varieties (Đekić et al., 2014, 2015). Several factors are decisive in increasing wheat yields: the cultivar, cultural practices, agroecological conditions, local climatic and soil characteristics, mineral nutrition and adequate protection from plant diseases, pests and weeds. Wheat productivity and grain quality in Central Serbia are governed by a range of factors, notably climate, soil, genetics and crop nutrition. Soil acidity in wheat fields in Central Serbia has become a severe problem that leads to a significant decline in grain yield and quality of wheat (Đekić et al., 2013, Jelić et al., 2012). Mineral fertilizers play a vital role towards improving crop yields but one of the main constraints in achieving proven crop potential is imbalanced use of nutrients, particularly low use of P as compared to N. The optimum rate of P application is important in improving yields of most crops (Jelić et al., 2013, 2014). In Serbia, farmers are using only nitrogen fertilizers for fodder crops while the use of P fertilizer is negligible. These crops are often grown on marginal lands. Hence, the production is low and quality is poor. The absence of record yields indicates that an answer could be sought in soil,

the main substrate for field crop production. Additionally, the major previous crops of small grains also suffer from negative nutrient balance. The use of incomplete production technology in previous decades had definitely affected the potential and actual soil fertility.
The objective of this study was to evaluate the effect of different fertilization systems on the grain yield and quality of wheat grown on a vertisol soil. The study was also aimed at optimizing fertilization for maximum profitability in the future wheat production of Central Serbia.

Materials and methods

Experimental design and statistical analysis

The study was carried out in a stationary field trial involving fertilization over a two years period from 2008/09 to 2009/10. Trials were first set up in the experimental fields of the Small Grains Research Centre in Kragujevac in 1970. Plot size was 50 m^2. The wheat cultivar used in the experiment was Vizija, the dominant cultivar in the production region of Serbia. This investigation included an untreated control and six variants of fertilization: NK_1 (80 kg/ha N, 60 kg/ha K_2O), NP_1 (80 kg/ha N, 60 kg/ha P_2O_5), NP_2 (80 kg/ha N, 80 kg/ha P_2O_5), NP_3 (80 kg/ha N, 100 kg/ha P_2O_5), NP_1K_1 (80 kg/ha N, 60 kg/ha P_2O_5, 60 kg/ha K_2O) and NP_2K_1 (80 kg/ha N, 80 kg/ha P_2O_5, 60 kg/ha K_2O). A non-fertilized variant served as a control. The trial was set up in a randomized block design with five replications. Fertilization was regular and followed a long-time scheme. Total amounts of phosphorus and potassium fertilizers and half the nitrogen rate are regularly applied during pre-sowing cultivation of soil.
On the basis of achieved research results the usual variational statistical indicators were calculated: average values, standard error and standard deviation. Statistical analysis was made in the module Analyst Program SAS/STAT (SAS Institute, 2000).

Agroecologial and soil conditions

This study was conducted over a three-year period in the Šumadija region, Central Serbia, on a Vertisol soil, at Kragujevac location, 173-220 m a. s. l. (44° 22′ N, 20° 56′ E), in a temperate continental climate having an average annual temperature of 11.5°C typical of Šumadija districts in Serbia and a rainfall amount of about 550 mm. Kragujevac area is characterized by a moderate continental climate, which general feature is uneven distribution of rainfall by month.
The data in Table 1 for the investigated period (2008-2010) clearly indicate that the years in which the researches were conducted differed from the typical multi-year average of Kragujevac region regard the meteorological conditions.

Table 1. *Mean monthly air temperature and precipitation (Kragujevac)*

Year	Months										Aver.
	X	XI	XII	I	II	III	IV	V	VI	VII	
Mean monthly air temperature (°C)											
2008-09	13.1	8.5	4.4	2.3	2.0	6.8	13.4	17.8	20.2	22.5	11.1
2009-10	11.7	8.8	2.6	0.9	3.2	7.2	12.1	16.5	20.2	23.1	10.63
Average	11.8	5.6	1.9	0.6	2.0	6.2	11.2	16.2	19.4	21.3	9.62
The amount of rainfall (mm)											
2008-09	31.3	30.6	29.7	57.7	76.9	40.3	16.8	46.0	137.8	25.2	492.3
2009-10	102.6	77.5	194.2	57.0	150.5	43.3	142.2	116.7	196.7	14.8	1095.5
Average	47.5	50.0	49.5	36.8	33.9	43.5	51.5	64.8	79.3	62.5	479.3

The average air temperature in 2008/09 was higher by 1.48°C and 2009/10 was higher by 1.01°C. The sum of rainfall precipitation in 2008/09 was higher by 13.0 mm, where the sum of rainfall in 2009/10 was 616.2 mm higher than the average of many years and with a very uneven

distribution of precipitation per months. Spring months April, May and June in 2009/10 were surplus of precipitation, what affected unfavorable on the crops. During the April in 2009/10 it was 142.2 mm of rainfall, what was 90.7 mm more compared with the perennial average. During the month of June in 2009/10 it was 196.7 mm of rainfall, what was 117.4 mm more compared with the perennial average. Regard the high importance of sufficient rainfall amounts during the spring months, particularly May for small grains production, the distribution and amount of rainfall over the growing season 2008/09 were considerably more favorable, what resulted with increment of yields during that year (Table 1).

The trial was set up on a vertisol soil in a process of degradation, with heavy texture and very coarse and unstable structure. The humus content in the surface layer of soil was low (2.22%). Soil pH indicates high acidity (pH in H_2O 5.19; pH in KCl 4.27), nitrogen content in soil is medium (0.11-0.15%), while the content of available phosphorus ranges from very low (1.7-2.9 mg/100 g soil) in the N and NK trial variants to very high (26.9 mg/100 g soil P_2O_5) in the NPK variants of fertilization. Available potassium contents are high, ranging from 19.5 to 21.0 mg/100 g soil K_2O.

Results and discussion

Results clearly show that yield components were significantly affected by fertilization (Table 2), the lowest values for grain yield and yield components were obtained in the untreated control.

Table 2. *Grain yield, 1000-grain weight and test weight of winter wheat*

Traits	Fertilization	Years						Average		
		2008/09			2009/10					
		$\bar{x}$	S	S_X	$\bar{x}$	S	S_X	$\bar{x}$	S	S_X
Grain yield, (t ha^{-1})	Control	2.341	0.456	0.204	1.412	0.532	0.238	1.876	0.677	0.214
	NK_1	3.056	0.777	0.347	2.556	0.604	0.270	2.806	0.707	0.223
	NP_1	3.106	0.261	0.117	2.484	1.480	0.662	2.795	1.054	0.333
	NP_2	3.068	0.414	0.185	2.379	0.913	0.408	2.724	0.761	0.241
	NP_3	5.404	0.317	0.142	3.724	1.242	0.555	4.564	1.231	0.389
	NP_1K_1	5.623	0.395	0.177	4.030	0.388	0.173	4.827	0.917	0.290
	NP_2K_1	5.134	0.5364	0.240	3.673	0.752	0.336	4.403	0.986	0.312
1000 - grain weight, (g)	Control	37.560	1.053	0.471	38.040	0.619	0.277	39.495	1.647	0.521
	NK_1	40.950	0.654	0.292	39.720	0.585	0.261	38.640	1.393	0.440
	NP_1	41.860	0.635	0.284	38.320	0.421	0.188	40.090	1.934	0.611
	NP_2	41.280	0.409	0.183	38.340	0.723	0.323	39.810	1.645	0.520
	NP_3	39.790	0.899	0.402	39.660	0.643	0.287	39.725	0.740	0.234
	NP_1K_1	40.130	1.059	0.474	39.480	0.687	0.307	39.805	0.909	0.287
	NP_2K_1	38.100	0.689	0.308	39.640	0.586	0.262	38.870	1.011	0.320
Test weight (kg/hl)	Control	76.380	2.162	0.967	73.210	2.071	0.926	76.055	3.312	1.047
	NK_1	78.900	0.400	0.179	73.370	1.647	0.736	74.875	2.408	0.762
	NP_1	79.450	1.724	0.771	72.930	2.390	1.069	76.190	3.958	1.252
	NP_2	79.040	1.485	0.664	74.050	1.470	0.657	76.545	2.976	0.941
	NP_3	78.720	1.781	0.796	73.170	2.216	0.991	75.945	3.485	1.102
	NP_1K_1	78.070	1.546	0.691	74.650	1.356	0.607	76.360	2.265	0.716
	NP_2K_1	78.640	1.207	0.540	72.850	3.382	1.513	75.745	3.879	1.226

Grain yield were the highest in the combined treatment with lime, manure and mineral NP_1K_1 fertilizer (4.827 t/ha). The grain yield of wheat significantly varied across years, from 2.341 t/ha to 5.623 t/ha in 2008/09, from 1.412 t/ha to 4.030 t/ha in 2009/10. During the first year of

investigations, the highest average value of 1000-grain weight achieved the NP_1 and NP_2-treatments (41.860 g and 41.280 g). During the second year of investigations (2009/10), the highest average value of 1000 grain weight achieved the NK_1 and NP_3 treatments (39.720 g and 39.660 g). A number of authors (Đekić et al. 2014; Jelić et al. 2013) underline that 1000-grain weight is a cultivar-specific trait, with considerably higher variations being observed among genotypes than among treatments or environmental factors. As illustrated in Table 2 during the first year achieved the highest test weight at NP_1-treatment (79.45 kg/hl), while the lowest test weight was the control treatment (76.38 kg/hl). During the second year of investigations, the highest test weight at NP_1K_1-treatment (74.65 kg/hl), followed by NP_2 (74.053 kg/hl).
Differences between grains yield of the untreated control and variants with fertilization were statistically highly significant. Fertilization with nitrogen alone resulted in a statistically highly significant increasing in grains yield, compared with the untreated control, but further increasing, when nitrogen was applied in combination with P and K fertilizers, did not result in statistically high significance. The study showed that among investigated fertilization variants the highest grain yields were achieved in variant with 80 kg/ha nitrogen rate, phosphorus rate of 60 kg/ha P_2O_5 and potassium rate of 60 kg/ha K_2O. Usage of fertilizers and certain amendments on extremely acid soils in certain years, particularly those less favorable for production, almost certainly had different effects on grain filling, resulting in diverse relationships between productive and qualitative traits. Presented results confirm the opinion of many authors that the traits analyzed are genetically determined, but strongly modified by the nutrient status and weather conditions (Đekić et al., 2014; Jelić et al., 2013).

Table 3. *Analysis of variance of the tested parameters (ANOVA)*

Effect of year on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(1. 68)	p-level
Grain yield (t ha^{-1})	19.9481	1.657779	12.033**	0.000911
1000-grain weight (g)	14.9503	1.819723	8.216*	0.005523
Test weight (kg hl^{-1})	436.7503	3.583620	121.874**	0.000000
Effect of fertilization on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(6. 63)	p-level
Grain yield (t ha^{-1})	13.15136	0.85347	15.409**	0.000000
1000-grain weight (g)	2.87295	1.92784	1.490	0.196039
Test weight (kg hl^{-1})	2.97970	10.51680	0.283	0.942780
Effect of the year x fertilization interaction on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(6. 562)	p-level
Grain yield (t ha^{-1})	20.62044	0.537467	11.154**	0.003755
1000-grain weight (g)	12.97490	0.511679	25.357**	0.000000
Test weight (kg hl^{-1})	4.23732	3.578286	1.184	0.328033

nsnon significant; *significant at 0.05; **significan at 0.01.

Based on the analysis of variance, it can be concluded that two are highly significant differences in grain yield regard the year of investigation (F_{exp}=12.033**) and significant differences at 1000-grain weight (F_{exp}=8.216*) and highly significant differences in test weight (F_{exp}=121.874**).
Highly significant differences in grain yield at investigated wheat cultivar were found relative to the fertilization (Table 3). In the dual interaction between the year and fertilization, significant effects were highly significant on grain yield and 1000-grain weight.

Table 4. *Correlation coefficients for the traits analyzed across treatments*

Correlations between the traits analyzed in the unfertilized control			
	Grain yield (t ha^{-1})	1000-grain weight (g)	Test weight (kg hl^{-1})
Grain yield (t ha^{-1})	1.00	0.69*	0.75*
1000-grain weight (g)		1.00	0.94**
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NK_1			
Grain yield (t ha^{-1})	1.00	-0.19^{ns}	0.21^{ns}
1000-grain weight (g)		1.00	-0.36^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_1			
Grain yield (t ha^{-1})	1.00	0.37^{ns}	0.29^{ns}
1000-grain weight (g)		1.00	0.79*
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_2			
Grain yield (t ha^{-1})	1.00	0.52^{ns}	0.50^{ns}
1000-grain weight (g)		1.00	0.95**
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_3			
Grain yield (t ha^{-1})	1.00	-0.04^{ns}	0.83*
1000-grain weight (g)		1.00	-0.22^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_1K_1			
Grain yield (tha^{-1})	1.00	0.23^{ns}	0.67*
1000-grain weight (g)		1.00	0.25^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_2K_1			
Grain yield (t ha^{-1})	1.00	-0.79*	0.79*
1000-grain weight (g)		1.00	-0.67*
Test weight (kg hl^{-1})			1.00

Positive correlations were observed (Table 4) between grain yield and test weight in all treatments. Pozitive and strong correlations were also found between grain yield and 1000-grain weight in the control treatment (r=0.69*) and negative and strong correlations in the NP_2K_1-treatment (r=-0.79*). Negative and strong correlations were also found between test weight and 1000-grain weight in the NP_2K_1-treatment (r=-0.67*). The present results confirm the opinion of many authors that the traits analyzed and their correlations are genetically determined but are strongly modified by the nutrient status of the environment and weather conditions (Jelić et al., 2013; Đekić et al., 2014).

Table 5. *Correlations between the traits analyzed during 2008-2010*

	Correlations in 2008/09			Correlations in 2009/10		
Traits	Grain yield, t/ha	1000-grain weight, g	Test weight, kg/hl	Grain yield, t/ha	1000-grain weight, g	Test weight, kg/hl
Grain yield (t ha^{-1})	1.00	0.28^{ns}	-0.03^{ns}	1.00	0.45*	0.27^{ns}
1000-grain weight (g)		1.00	0.47*		1.00	-0.07^{ns}
Test weight (kg hl^{-1})			1.00			1.00

Testing the correlation coefficients between grain yield and 1000-grain weight of wheat (Table 5) was found positive and medium-dependent statistically average correlation between test weight and 1000-grain weight in 2008/09. However, 1000-grain weight were significantly positively correlated with grain yield only in the second year ($r=0.45^{*}$). Test weight in 2008/09 was negatively correlated and 2009/10 was positively correlated with grain yield. The results suggest that grain yield and quality formation is affected by both genetic and environmental factors (Jelić et al., 2013).

Conclusion

Over the two-year period, all investigated treatments of wheat achieved the highest average grain yield in the NP_1K_1 variant of fertilization with the higher rate of phosphorus (4.827 t/ha). Considering the average yields value and test weight in two-year period, it was evidently that the yields and test weight were highly statistically significantly different and 1000 grain weight were significantly different between the year. Effect of fertilization on the grain yield were highly statistically significantly. Significantly positively correlated with yield and 1000 grain weight both in 2009/10 (0.45^{*}, respectively), and positively correlated with 1000-grain weight and test weight both in 2008/09 (0.47^{*}, respectively). Significant positive correlations were observed between 1000 grain weight and test weight in control, NP_1 and NP_2 treatments, and negatively correlations were observed between 1000 grain weight and test weight in NP_2K_1.

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FORTIFICATION AND BIO-AVAILABILITY OF MINERAL ELEMENTS FROM AGRICULTURAL PLANTS

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Abstract

Trends for increased biomass and grain yield production with costs lowering, has consequence in products low in mineral nutrients. Minerals deficiencies in nutrition, particularly of iron, magnesium, zinc and selenium are present worldwide. To combat such a situation, increase in mineral nutrients in food is necessary. This could be provided by fortification which includes different cropping practices and genetic engineering. Almost half of soils are deficient in mineral nutrients. Cropping practices which include increase in soil fertility, application of foliar fertilizers etc. could be measures for increasing plant’s ability to utilize mineral elements. Methods of conventional breeding and genetic engineering could increase mineral nutrients concentration in edible parts of plants. Fortification also includes lowering of antinutrients (phytate, phenolics, etc.) that inhibit nutrient bio-availability, as well as increasing of enhancing substances - promoters (ascorbic acid, β-carotene etc.) that promote nutrient bio-availability. Increase of mineral nutrients concentration and their availability from produced crops are complex traits which demand new approach in crop production.

Key words: *crop improvement, mineral elements, bio-availability, antinutrients, promoters*

Introduction

Nutrients, as a basis for wellbeing came from agricultural production. If agricultural systems is not capable to product enough food diversity and quantity to satisfy all the nutrients essential to human life, people will suffer, mortality and morbidity rates will increase, working productivity will be reduced, societies will deteriorate (Welch, 2002). In recent time, many food systems, irrespective that they belong to developed or developing countries are incapable to meet the nutritional needs. Nutritional deficiencies afflict over three billion people globally, causing chronic diseases, such cancer, cardio-vascular diseases, stroke, diabetes, osteoporosis etc. (Graham et al., 2007).

Agricultural production depends mainly on variation in environmental factors. In combination with low soil fertility, soil degradation, inadequate use of fertilizer inputs climatic factors could be the main reason for food insecurity, (St.Clair and Lynch, 2010). One of the most important factors that induce elemental imbalance in plants is raising CO_2 level in atmosphere. Fernando et al. (2012) obtained that elevated atmospheric CO_2 reduced proteins and mineral elements concentrations in wheat grain by 12.7%.

The accumulation of mineral elements in plant tissues is limited by their supply and availability from soil, unless foliar fertilizer was applied (White and Broadley, 2009). Adequate mineral nutrition is crucial when crops are growing on infertile soils. Physical characteristics of some soil, like concretion, poor drainage, shallowness, poor water retention, as well as chemical characteristics, such as acidity, alkalinity, salinity, ion toxicity and sub-optimal availability of mineral nutrients, could be stressful to plants. Most of the soils worldwide are characterized by low availability of mineral nutrients and/or ion toxicities. This

includes the combination of low P, low Ca, Al toxicity, and Mn (Lynch and St.Clair, 2004). Imbalance between ions is present in areas with intensive agricultural production, where high yielding potential of grown crops, together with high inputs of N, P, K fertilizers devastate soils for other mineral nutrients. Also, in areas with poor fertilization level, even good soil fertility was decreased. The aim of this study is emphasize importance of biofortification in improvement of global health.

Importance of mineral nutrients for human health

Nutrition is source of all substances necessary for human organism. Nutritional deficiencies, which mainly include Fe, Zn, I, Se, vitamin A, affect almost 3 billion people worldwide and are responsible for almost two-thirds of the childhood deaths worldwide (Graham et al., 2007).

Fe deficiency, recognized as anemia, affects about 30% of the world's population. Poor Fe availability from foods, is the main factor that induce anemia, while the other factors, such deficiencies in Zn, I, Se, vitamin A and its pro-vitamin β-carotene, folate, vitamin B12, certain gut bacteria, and pathogens in human organism could contribute to anemia, too (Graham et al., 2007). Zn deficiency has been connected to abnormal blood and muscle mass losses, problems associated with other physiological processes due to its high physiological requirements for growth, puberty, pregnancy and lactation (Hunt2003). This means that Zn deficiency is ultimate "hidden hunger", affecting about 2.6-3 billion people (Graham et al., 2007). Mn is essential for humans. It has important role in antioxidant system for metabolism, bone development and wound healing.

Ca plays an important role in skeleton structure and its health, and it is also important for cardio-vascular system, kidneys, regulation of energy metabolism, (inhibiting diet-induced obesity, promoting lipolysis, lipid oxidation, and thermogenesis) (Cappuccio et al., 2000; Zemel, 2004). Mg have many important functions in cellular metabolism and homeostasis, including transport of K and Ca ions, modulation of signal transduction, energy metabolism and cell proliferation (Saris et al., 2000). Low Mg status has been associated with a chronic inflammatory stress component, widely connected with obesity, alcoholism, atherosclerosis, eclampsia, hypertension, osteoporosis, diabetes mellitus, cardiac diseases, asthma, and cancer (Saris et al., 2000; Nielsen, 2010). Se has structural and regulatory role as constituent of whole range of selenoproteins, it plays an important role as an antioxidant and catalyst for the production of active thyroid hormone (iodothyronine 5'-deiodinases) (Rayman, 2000). The other important functions are connected to the proper functioning of the immune system, regulation of the inflammatory mediators in asthma. I deficiency causes mental defects, goiter, reproductive damage, childhood mortality, and hypo- and hyperthyroidism (Vitti et al., 2003). To combat I deficiency, universal salt iodisation is introduced as mandatory measure in nearly all countries worldwide and two-thirds of the world's population is covered by iodised salt. Still one-third of households lack access to adequately iodised salt (Andersson et al., 2010).

Mineral nutrients from various foods are partly available owing to the presence of anttinutrients, like phytic acid, phenolic compounds, some types of cellulose etc. that inhibit absorption. Other substances, promoters, increase absorption of nutrients, diminishing negative effects of antiunutrients. From this point Graham et al. (2007) statement "You are not what you eat, you are what you eat and do not excrete" is referred to bioavailability.

Biofortification

Biofortification is sustainable strategy to combat micronutrient malnutrition. It combines different techniques, like plant breeding and cropping measures. Breeding is using to improve genotypes with enhanced efficiency for absorption and accumulation of targeted nutrients.

Agronomic fortification includes mainly usage of soil and foliar fertilizers (with increased concentration of desired nutrients) and techniques that enhance absorption of mineral nutrients.

Modern crop production has to be based on genotypes able to achieve high yields, with lesser inputs of water, fertilizers, agro-chemicals etc. They also have to have improved nutritional quality, to meet nutritional requirements of humans and animals. The useful breeding techniques for fortification are conventional breeding, marker assisted selection (MAS), which could also be combined with transgenic approach. Conventional breeding is possible only if high variation among genotypes is present in gene pools that control desired trait and if it is established in nutrient efficiency in the field Rose et al. (2013). Dragičević et al. (2013) and Gupta et al. (2015) pointed that maize is characterized as a species with high variability in β-carotene, Fe, Mn and Zn and so, some antinutrients, offering distinct opportunity to deliver bioavailable minerals through diet. According to chemical composition, three distinct strategies are being distinguished: I - to increase mobility of mineral nutrients together with grain binding capacity; II - to decrease inhibitors of absorption; III - to increase concentration of substances that directly promotes absorption or indirectly by stimulation of gut's microbiome (Clemens, 2014). Up today majority of efforts was given to the firs group.

Introduction of genetically modified plants gave opportunity to overcome problem of boundaries in natural variation in accumulation and bioavailability of mineral elements. Overexpression of uptake and storage proteins could increase Fe and Zn concentration to a limited extent, so introduction of substances that enhances mineral element absorption in gut even in the presence of naturally occurring inhibitors could provide satisfactory results (Lonnerdal, 2003). That is why strategies for increase in concentration of important nutrients, like β-carotene, vitamin C, or folate are important, too. Genome-wide association studies were used to identify variation for genes controlling enhancing substances, like β-carotene (Suwarno et al., 2015), resulting in several fold higher β-carotene level in modified plants (such "golden rice"). Biofortification of plant folate resulted in 25-fold more folate accumulation in tomato fruits (Garza et al., 2007). Interesting example is simultaneous modificatrion of three separate metabolic pathways in maize, resulting in kernels that contained 169-fold higher amount of β-carotene, 6-fold higher amount of ascorbate, and two-fold higher amount of folate (Naqvi et al., 2009).

Even if crops efficiency is enhanced, the nutrient absorption from soil that is low in mineral nutrients is under question. Crops, like humans also require balanced nutrition for optimal growth and development. The application of fertilizers, as fortifying measure could improve nutritional quality of crops, as well as to increase yield. For instance, Zn-enriched NPK fertilizers could full support on-going breeding programs with genotypes with increased Zn efficiency (Cakmak, 2008). Fertilizers that contain macro-elements (N, P, K, S) could affect absorption and accumulation of microelements. For instance, application of N fertilizers in rice increased transportation ability of microelements from root to shoot (Hao et al., 2007). Due to the variations in plant requirements during vegetation, variations in environmental factors, and possible runoff, the combination of soil and foliar fertilizers could give the best results in Zn enrichments (Cakmak, 2008). Prolonged effect of these measures is obtained in higher vigor of enriched grains, denser stands and higher stress tolerance on potentially Zn-deficient soils.

Application of foliar fertilizers is the most efficient way for utilization of deficient mineral nutrients, since soil factors which restrain availability of some mineral nutrients are diminished. Moreover foliar fertilizers that contain different bio-active compounds, like secondary metabolites and phytohormones could enhance Fe accumulation in soybean grain, also increasing potential availability of Fe, Mn and Zn to humans and animals (Dragičević et al., 2015a). Some of the useful methods for enrichment of crops with Se is use of plants

grown on Se rich soils (part of phytiremediation) as a green manure or supplementary source (Wu et al., 2015). Sustainable agricultural technologies are based on natural potentials to improve agricultural products. Very important place was given to the application of biofertilizers, like arbuscular mycorrhizal fungi which facilitate absorption of Cu, Fe and (to a limited extent) Mn. Also, phytosiderophores from maize root could improve the Fe nutrition of intercropped peanut and soybean (Xiong et al., 2013, Dragicevic et al., 2015b), with increased potential bioavailability of Mg, Fe and Zn from grain of maize and soybean, due to the elevation of β-carotene, together with reduce in concentration of anti-nutrients in grain.

Agronomic biofortification is required for optimizing and ensuring the success of genetic biofortification of cereal grains (Cakmak, 2008), while the efficiency of all applied cropping measures in increasing concentration of mineral nutrients depends on many factors, like soil type, crop, cultivar, rotation, environmental and other factors, resulting in a specific set of measures for individual regions (Rengel et al., 1999). Besides, usage of fortified plants in nutrition lower the risk of overdosing. Just increase in concentration of mineral nutrients in edible parts of plants doesn't mean that they will reach the goal, to enable health benefit through nutrition. Different techniques, like conventional breeding, MAS and genetic modifications, are using to increase bioavailability of mineral nutrients due to the decrease of antinutrients, mainly phytic acid concentration in grain. The molar ratios between mineral nutrients with P are useful traits in biofortification research (Šimić et al., 2012). The mutations that block PA synthesis, contributes to the increased P bioavailability, due to increased concentration of inorganic P (Raboy, 2013). Owing to negative aspects in PA decrease in grains, such reduced germination, emergence, tolerance to pathogens and various diseases, and yield, different processing methods could be useful in decrease of PA, like cooking, germination, fermentation, soaking, autolysis (Urbano et al., 2000). When anti-carcinogenic properties of PA are also taken into account, strategy for increasing promoter concentration (such vitamin C, β-carotene and nicotianamine), could express the promising results for increased bioavailability. For instance, introduction of nicotianamine synthase gene (OsNAS1) into rice significantly increased nicotianamine concentrations in both unpolished and polished grain, increasing twice Fe bioavailability (Zheng et al., 2010).

Conclusion

Raising population, increased CO_2 concentration in atmosphere, together with meteorological extremes, soil devastation, as well as crops with increased yielding potential contributes to globally present malnutrition. To combat such situation, biofortification, as innovative as sustainable strategy arose. Breeding and genetic engineering produced new genotypes, with improved efficiency in absorption and accumulation of mineral elements in edible parts of plants, even from the soils poor in nutrients. Agronomic biofortification is focused on environment, i.e. application of measures that enhance absorption of mineral elements. Special attention was given to fertilization through soil or plant foliage, assuring fast metabolisation of targeted mineral nutrients. Combination of agronomic fortification and breeding offers reliable results in biofortification. It also includes breeding or modification of genotypes with decreased antinutrients concentration, as well as increased concentration of promoters, enabling better availability of mineral nutrients to humans and animals, as a final goal. The holistic approach that includes application of the results from different scientific areas could be fruitful as more effective and sustainable biofortification solutions and improving of human health.

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EFFECT OF BENTAZONE ON WHEAT GENOTYPES DIFFERENT BY Lr GENES GROWTH AND PARASITES IN HARD CONTINENTAL CLIMATE

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Abstract

Six wheat varieties where tested on the effect of 2,1 kg/ha bentazone in herbicide at field trial. Stem growth two weeks after treatment at heading and flowering was -5- 9% due to its accelerated degradation correlated to hydrolytic stability of race specific leaf rust resistance genes (Lr). Of variety Pobeda was -2, increasing to -7% in June 2015th. At additional Lr near isogenic lines (NILs) different by nonspecific Lr genes present also in the tested varieties grown in greenhouse at 20^{o}C during December, assumed plant parts lengths reduction estimated ten days from first leaves treatment was -17-20% similar to Pobeda of -15%. Than, of specific Lr genes about halved influence could be related to increased amount of Mg in seed solving consequences of degraded herbicide in chloroplast membrane where relisted SO_2 transfer in acids that reduced chlorophyll. 23 – 28th May, temperature below 15^{o}C and heavy rains facilitated *Puccinia striiformis* and facultative parasites infections. *Septoria tritici* growth at middle leaves was reduced for 10% causing 10% of yellow rust increase at susceptible varieties equal by 5% *Pyrenophora tritici repentis* main *Puccinia triticina* antagonist which toxin such effect in June was not disturbed. Reaction types to *Puccinia triticina* after treatment were increasing, except stable maximal one at Pobeda. Scab of 2% was generally reduced to about 1%. Shortened currency period of approximately two months by Lr genes 1, 3a, 19, 24 and 29 facilitated multiple pests protection regional suggested after continual moisture at flowering when over frequented all of weeds appeared.

Key words: wheat protection, bentazone, Lr genes

Introduction

The attempts to solve problem of negative consequences of herbicides to wheat were trough genes mutations. Of bentazone adequate one was achieved by Gamma ray irradiation while mutation was recognized throw ten times increased dose and herbicide concentration in the middle parts of leaves instead of all round effect (Kim et al., 2007). By same strategy founded was tolerant gene to glyphosate (Gordon et al., 2004) while of tolerant soya analysis confirmed linkage by phosphate group to novel gene (Funke et al., 2006). Such, problem of consequential former residua degradation was not solved. Increased rust resistance of wheat and soya bean genotypes declared as to glyphosate resistant was recognized (Anderson et al. 2006, Feng et al. 2005). Initiated were investigations focusing accelerated enzymatic degradation of pesticides by structure similar to of *Puccinia triticina* cell wall (Harder and Chong, 1984). Recognized were accelerated degradations of dithiocarbamate, phtalamide, neonicotinoides and glyphosate at seedlings by decreased growth in the presence of same of specific Lr (leaf rust resistance genes) in near isogenic lines (NILs) or wheat varieties as well as confirmed simultaneous influence of products on *Puccinia triticina* reaction type. Lr genes products were founded to be different by hydrolytic stability, cleaved linkage strength and approach ability to middle weighted elements sulphur, phosphorus and chlorine (Jerkovic and Prijić, 2012; Jerković et al., 2014, 2015).

The hard continental climate characterized the highest amplitude between winter and summer temperatures amplitude more than 50^{O}C during wheat vegetation. When combined pesticides were applied twice in spring yellowing of leaves of the varieties except Pobeda was linked to

interactions between specific Lr genes and pesticides and simultaneous accelerated degraded gluten in leaves (Jerkovic et al., 2014a). In the same paper assigned was problem of defining the stable acidic elements amount applicable trough pesticides in focused climate with grain filling period about forty days. Simultaneous of glyphosate influence on *Septoria tritici* facultative parasites in field was recognized as well as no significant influence to *Pyrenophora tritici repentis (Ptr)* growth confirming results of Sharma et al. (1989). By forecasting model for the first time involving of the facultative and obligate parasites antagonism recognized was time for economical protection at the beginning of June throw reduction of *Puccinia triticina* at susceptible varieties when Ptr intensity at middle leaves was bellow 10% (Jerković and Jevtić, 2012; Jerković et al., 2013). Because of short grain filing period and lower yields once protection by pesticides from more pests become only rentable solution also. The problem of weeds and *Septoria tritici* was not generally solved with glyphosate because of Lr genes specificity according to activation time in drought conditions and possible consequences as were on weeds. Bentazon was found to be contact herbicide acting as a photosynthetic electron transfer inhibitor. Its selectivity was previous related to the ability of the crop plants to quickly metabolize bentazon to 6-OH- and 8-OH-bentazon and conjugate it with sugar (Banokowska, 1999). Fast linkage to membrane of chloroplast reducing effect instead of enzyme inhibiting as was by glyphosate, recognized by Bethenfalvay et al., (1979). Uptake and accumulation of bentazones was investigated by Sterling et al. (1990) while transformation to lipids was not recognized in soybean and Abutilon tissue cultured cell suspension. Photo catalyst based on Zn was recognized by Borhade et al. (2014). Similar could be explained detoxification in rice (Yingzhi et al., 2015) generally founded to be crop damaging, immediately after treatment. Rice non host resistance to rusts (Aylife et al., 2011) was previous related to collase fast consequence, hydrogen peroxide relisted before haustorium formation. Early at spring application suggested Bentazone manufacturers focusing under allowed original residua amount in wheat seed and effect on branching weeds. By its structure it was expected that race specific Lr genes from most of clusters could accelerate relist bentazone sulfur linkages. Hypothetic were different interactions of nonspecific and specific Lr genes in regional varieties with bentazone and parasites in field.

Aimed was to recognize not so risky solution for occasional economical treatment in hard continental climate, focusing all weeds reduction after appearance, as well as Scab and *Septoria tritici* without disturbed *Pyrenophora tritici repentis* antagonism to leaf rust cause.

Material and method

Six wheat varieties were selected according to different combination of specific and nonspecific Lr genes (Jerkovic et al., 2013). These varieties and four Lr NILs with only nonspecific Lr genes were grown in the glasshouse at average air temperature around 20^{o}C at day/night regime 10/14 h. 50 seeds of each genotype was simultaneous sown in pots (5x5x7cm). Daily watering of the soil was stopped two days before 0,21 mg/m2 of pure bentazone in herbicide in 0,1 l of water suspended was applied six days after germinating. NILs were halved by cover before the treatment. For further calculations criterion was that stems of NIL sample could to be different in the interval of 5 mm. At least five plants from twenty viable in each of the replications had to be in representative sample for calculation of average part lengths of the NILs. Of treated and control NIL growth potential was equalized across formula: (average first and second leaf lengths sum of treated or control NIL with lower average stem length) x average higher stem length: average lower stem length + average higher stem length while growth difference presented in percents (average up ground length of treated plant parts: average up ground length of treated plant parts x 100).

The field trial was postulated according to method of Jerkovic and Jevtić (1997). Such, the control and treated varieties again by 2,1 kg –ha bentazone at 30th April , 15th May. and 3rd June 2015th were sown parallel at six m^2, 29.10.2014th while distanced two. The estimation of *Septoria tritici, Purenophora tritici repentis intensity* presented in percents of covered area at middle leaves was performed at 17th May while 8th June at last two. Simultaneous estimated was also reaction type (Stakman et al., 1962) to *Puccinia triticina* for the difference of *Puccinia striiformis* when was only intensity at flag leaf. The Scab intensity was presented trough percent of necrotic middle of the spikes while parasite proved by development in permanent humid conditions kept in Petri dishes at air temperature around 20^{O}C. At 3th June were measured the distance of spike from second internodia below and stem length while their ratio was SAGR.

Results and discussion

At seedling stage of Lr near isogenic lines (NILs) with Lr 34, Lr 13 present also in the tested varieties parts lengths reduction by bentazone was -17-20% similar to Pobeda one of -15%. Specific Lr genes in other varieties caused reduction 3-7%. At defined seedling stage nitrogene was not uptaken from the soil due to reserves in seed (Vuurde and Tonneyck, 1978). The vigour by bentazone was not recognized as when thiametoxam was applied because of relative lover amount of fast viable nitrogene from pesticide (Jerkovic et al., 2015). The bentazone similar dosed and applied in field did not caused spike whitening as glyphosate (Jerkovic et al., 2014a). The previous recognized lower influence on growth than of glyphosate in the presence of specific Lr genes could be related to additional Na in the particular applied herbicide recognizable by brown color according to EPA, that decreased Mg salted amount. In Table 1 presented was treatment results when recognized were highest differences in growth. Exceptional opposite was recognized at variety Pobeda related to no presence of specific Lr genes confirmed by unique SAGR also. Generally stem reductions were correlated with these at seedling stage while focusing nonspecific Lr genes in near isogenic lines trough Pobeda, differences were not so huge and related to assumed prolonged daily light and intensity during the May reducing the effect of herbicide. The most of the varieties expressed SAGR values bellow of Pobeda one were related to presence of sucrose (Lr 13) and SUTs (Lr 34) and specific Lr genes (Jerkovic et al., 2013a), however excluding sugar as tolerance cause Amylase was recognized as product of Lr 22b found to be in Rapsodia accompanied with sucrose trough relative highest SAGR up to 0,68. Generally, parameter was correlated with previous years but lower for approximate 0,05 than maximal one because of relative lower temperatures during the May. By its declining the infection efficiency of obligate parasites was relative increased. Of both facultative leaves affecting ones growth was increased but fructification absent by treatment except stable from all leaves of Rapsodia. The intensity at upper leaves of controls was declining from maximal of 20% correlated to SAGR as was recognized previous by Jerkovic and Jevtić (2012). Focusing *Puccinia triticina*, of nonspecific Lr genes enzymes removed or degraded sugar simultaneous eliminated it as a cause of whitening about infection sites when bentazone based herbicide was applied. Whitening without pustules was recognized at most of the treated varieties at 17th May except on Pobeda with stable maximal reaction type. Moderately resistant reaction type was explained by effect on bentazone and overcome resistance of Lr 1 in Simonida and Rapsodia. The all of reaction types on flag leaves were maximized at June confirming no further presence of herbicide residua with sulfur. Across decreased infection severity to leaf rust opposite than was of yellow one variety Enigma had to have Lr 19 beside previous detected Lr 29 trough interactions with pesticides. It was not disturbed spreading on upper leaves of facultative parasites from Rapsodia with reproductive organs formed before treatment related to accelerated growth of further plant parts defined by increased SAGR. The

growth at lower leaves of *Septoria tritici* was stopped by herbicide. These parasite toxins also influenced yellow rust at flag leaves which development was facilitated by unusual period of stable temperatures 10-15^{0}C from 23-28th May. Its reduction was for 10% due to 10 % increased growth of *Septoria tritici* at controls focusing varieties without Lr 3a as was in NS 40S and Lr 24 in Ilina. Scab intensity was halved by both treatments most likely by not complete entrance of herbicide concluded trough same effect on Pobeda and varieties containing Lr 1 permanent active during critical time for the infection.

Table 1. The disease causes intensity changes by bentazone and growth reductions at adult and seedling stages

Variety	Pyr.tritici repentis T K 17th May		Septoria tritici at middle leaves T K 17th May		Puccinia striiformis T K 8th June		Puccinia tritici T K 8th June		Stem reduction 17th May	3rd June	Growth reduction of seedlings	SAGR
Pobeda	5	10	20	30	30	20	50	40	-2%	-7%	-15%	0,60
Simonida	5	5	10	20	30	15	40	30	-9%	-5%	-6%	0,64
NS 40S	5	5	20	20	5	t	30	20	-5%	-3%	-6%	0,58
Ilina	5	5	10	15	5	t	30	20	-3%	-3%	-5%	0,58
Enigma	5	5	20	30	20	15	20	15	-5%	-1%	-3%	0,60
Rapsodia	10	10	15	25	15	5	15	10	-5%	-2%	-7%	0,68

Legend: K= control plants, T= treated plants

Main antagonist of leaf rust *Pyrenophora tritici repentis* (Jerkovic et al., 2005) was present mostly about 5% at middle leaves. In June its spreading on upper leaves was recognized in traces about Rapsodia surrounded varieties while development was not influential on leaf rust cause because of no time for enough toxins production from lessons appeared in lower density than were below. Each of toxins from two facultative parasites influence to reduction of yellow rust cause was still not precise defined but seemed to be as to leaf rust one (Jerković, 2008).

Conclusion

Of bentazone residua with sulfur were degraded after later than proposed treatment facilitated by some of different clustered specific Lr genes products concluded across no effect on leaf rust cause in June and increased growth two weeks after treatment. There was no complete equalizing about half of grain filling period. The advantage from treatment at wheat flowering was recognized trough reduction of later appeared weeds, than predictable Scab by temperatures and humidity and *Septoria tritici.* The treatment against last mentioned parasite in reproductive phase had no sense while antagonism between *Pyrenophora tritici repentis* and leaf rust cause was not disturbed. The effects of treatment on obligate parasites reduction had to be slight increased in production at varieties with effective specific Lr genes because of induced complete resistance in May and not so nearby infection source.

The residua of bentazone when sulfur was relisted were recognized to be degraded in underground water by micro organisms. However, the residua facing directly soil surface had to be reduced by later treatments as well as micro organism’s development supported by cultural plants parts and weeds after their incorporation.

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THE EFFECT OF PRUNING SYSTEM ON VARIATION OF NUMBER OF BUNCHES OF THE VINE CULTIVAR WHITE TAMJANIKA IN ZUPA VINE DISTRICT, SERBIA

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Abstract

This paper illustrates the variation of the number of bunches in the cultivar White Tamjanika. The investigation has been carried out at a productive vineyard in private property, at the location Svracak, during the period 2008-2010. The vineyard was planted in 2002, with planting distance 2.8 x 0.9 m. Three pruning variants with short and long spurs and three variants of mixed pruning with canes eight and ten buds long were applied. Ecological conditions were favorable for growth and development of vine cultivar White Tamjanika. The study was aimed to variation of the number of bunches of this cultivar, as well as possibility for its spreading in the vine district of Zupa. The following parameters were observed: bunches per bud, bunches per developed canes, bunches per productive canes and bunches per vine. Results of the study confirm a possibility of successful growing of White Tamjanika cultivar in the conditions of Zupa Vine District.

Key words: *cultivar, bunches, ecological conditions.*

Introduction

The Muscats are a large, wide-spread family of grapevines, having in common besides the name the typical Muscat flavour. A huge number of synonyms and homonyms makes it difficult to identify them (Crespan and Milani, 2001). Bešlić et al. (2012) researched genetic profile of 12 varieties through a genotyping approach that included the „core set" of 9 SSR markers chosen by the international grape comunity for genetic identification and further 13 common microsatellites to strengthen relationship analysis. Consistent matching with SSR markers of grapevines cultivated in neighbouring countries or maintained in European germplasm collections was found for most of the molecular profiles, suggesting possible synonyms. These included three Muscat-type cultivars which showed identical SSR profiles with true-to-type "Moscato Giallo", "Moscato Rosa" and "Muscat fleur d'Oranger" varieties. When compared with germplasm representing the classical eco-geographic grouping of grape varieties, Serbian cultivars were divided into two distinct clusters. The Muscat-type cultivars were included within the Convar orientalis subconvar caspica.

This paper has been aimed to establish the most important agrobiological traitis of White Tamjanika cultivar. Environmental conditions significantly influence the biological characteristics of the vine, as stated by many authors: Buric et al. (1985), Todic et al. (2000), Rapcha (2001), Cirkovic et al. (2013). Environmental conditions of Zupa vine district enable a normal annual development cycle of vine, as well as good ripening of the studied cultivar grapes and shoots.

The applied pruning systems and fertile bud loads per vine expressed a significant effect on variation of the number of bunches per bud, bunches per developed cane, bunches per productive cane and bunches per vine.

Based on the analysis of the obtained results one can conclude that the applied pruning systems expressed a significant effect on number of bunches of the vine cultivar White Tamjanika in the conditions of Zupa vine distrct.

Material and Methods

The investigation has been carried out during the period 2008-2010 at a productive vineyard in private property, at the location Svracak. This vinyard is located in Župa vine district, characterized by moderately parish climate with average annual air temperature of 11.0^{o}C and average vegetational air temperature of 16.8^{o}C. The absolute minimum of air temperature during the study was -14.0^{o}C. The absolute maximum of air temperature during the study was 36.5^{o}C. The sum of active temperature was slightly higher than the multiannual average (3528^{o}C) and varaied from 3543^{o}C (2010) to 3673^{o}C (2009). The average annual precipitation amount in the observed period varied from 619.5 mm (2008) to 738.3 mm (2009), and from 380.6 mm (2008) to 491.4 mm (2010) of which fell during vegetation. Agroecological conditions in the observed period were very good for the cultivar White Tamjanika.

The vineyard was established in 2002 and it is in the period of growing yield. The planting distance of 2.8 x 0.9 m (3968 vines per ha). Bud load per vine was varied from 18 to 36 buds in six variants. Three pruning variants with short and long spurs and three variants of mixed pruning with canes eight and ten buds long were applied(tab 1.). The trial was set in random complete block design with four replications, and the data were processed by analysis of variance.

Table 1. Review variants and number of buds per vine

No	Variants	Pruning system	Number of buds per vine
1	I	(6 X 3)	18
2	II	(4 X 4 + 4 X 2)	24
3	III	(4 X 5 + 3 X 2)	26
4	IV	(2 x 10 + 3 X 2)	26
5	V	(3 X 8 + 3 X 2)	30
6	VI	(3 X 10 + 3 X 2)	36

Results and Discussion

The number of bunches per vine is the ratio real fertility, and shows the average number of bunches obtained from pruning abandoned buds. The highest number of bunches per bud (tab. 2) was observed in the five and six variants with the highest load (1.26), and the lowest in the second variants with the lowest load (1.12). The fertile bud loads per vine expressed a significant effect on the number of bunches per bud. By leaving a larger number of buds per vine the number of bunches per bud developed decreases in the first and second variants.

Table 2. The number of bunches per bud

Variants	Years of invesigation			Average
	2008	2009	2010	
I (6x3)	1.28	1.28	1.17	1.24
II (4x4 + 4x2)	1.12	1.14	1.11	1.12
III (4x5 + 3x2)	1.18	1.18	1.14	1.17
IV (2x10 + 3x2)	1.26	1.21	1.20	1.22
V (3x8 + 3x2)	1.26	1.28	1.23	1.26
VI (3x10 + 3x2)	1.25	1.30	1.24	1.26
Average	1.22	1.23	1.18	1.21
CV%	15.20	15.20	14.06	14.88
F-test	Years (A)	Variants (B)	AxB	
	1.34	2.94*	0.20	
LSD 0.05	Years (A)	Variants (B)	AxB	
	0.06	0.09	0.16	
LSD 0.01	0.09	0.12	0.21	

The number of bunches per developed cane the capacity index (tab. 3). The values of this indicator varied significantly depending on weather conditions of the investigated year. The short pruning had a positive effect on the number of bunches per developed cane, indicating that the buds at the base of shoots vary birth. To access such data came Garić (1997). The highest number of bunches per developed cane was in 2009 (1.42), very significantly higher than in 2010 (1.36), which recorded the lowest number of bunches per developed cane. Between 2008 and 2009. capacity index did not vary significantly. The number of bunches per productive cane the fertilitiy (tab.4). The highest number of bunches per productive cane was observed in the sixth variants (1.72), and the lowest second variants (1.40). The number of bunches per productive cane is quite a variable feature, which is very dependent on the agroecological conditions (Nakalamic, 1987).

Table 3. The number of bunches per developed cane

Variants	Years of invesigation			Average
	2008	2009	2010	
I (6x3)	1.39	1.42	1.27	1.36
II (4x4 + 4x2)	1.23	1.26	1.23	1.24
III (4x5 + 3x2)	1.42	1.44	1.40	1.42
IV (2x10 + 3x2)	1.42	1.38	1.38	1.39
V (3x8 + 3x2)	1.46	1.49	1.43	1.46
VI (3x10 + 3x2)	1.46	1.52	1.47	1.48
Average	1.40	1.42	1.36	1.39
CV%	14.37	15.60	15.49	15.17
F-test	Years (A)	Variants (B)	AxB	
	1.095	5.561**	0.258	
LSD 0.05	Years (A)	Variants (B)	AxB	
	0.07	0.10	0.18	
LSD 0.01	0.10	0.14	0.24	

Table 4. The number of bunches per productive cane

Variants	Years of invesigation			Average
	2008	2009	2010	
I (6x3)	1.52	1.51	1.37	1.47
II (4x4 + 4x2)	1.40	1.43	1.38	1.40
III (4x5 + 3x2)	1.58	1.59	1.64	1.60
IV (2x10 + 3x2)	1.58	1.53	1.56	1.55
V (3x8 + 3x2)	1.57	1.60	1.54	1.57
VI (3x10 + 3x2)	1.67	1.76	1.74	1.72
Average	1,55	1,57	1,54	1,55
CV%	12.47	16.13	18.55	15.83
F-test	Years (A)	Variants (B)	AxB	
	0.273	6.913**	0.389	
LSD 0.05	Years (A)	Variants (B)	AxB	
	0.08	0.12	0.20	
LSD 0.01	0.11	0.16	0.27	

The obtained results show that the dependence the number bunches per vine on the fertile bud loads per vine assessed based on the data shown in table 5. The number buds per vine a positive effect on the number bunches per developed cane. By leaving a larger number of buds per vine during pruning 18 buds (variants I) to 36 buds (variants VI) the number of bunches per vine increased from 22.37 to 45.47.

Table 5. The number of bunches per vine

Variants	Years of invesigation			Average
	2008	2009	2010	
I (6x3)	23.00	23.10	21.00	22.37
II (4x4 + 4x2)	26.90	27.40	26.60	26.97
III (4x5 + 3x2)	30.60	30.70	29.60	30.30
IV (2x10 + 3x2)	32.70	31.40	31.30	31.80
V (3x8 + 3x2)	37.90	38.40	36.90	37.73
VI (3x10 + 3x2)	44.90	46.70	44.80	45.47
Average	32.67	32.95	31.70	32.44
CV%	26.45	27.56	27.64	27.12
F-test	Years (A)	Variants (B)	AxB	
	1.115	86.725**	0.147	
LSD 0.05	Years (A)	Variants (B)	AxB	
	1.73	2.45	4.25	
LSD 0.01	2.29	3.24	5.60	

Conclusion

Based on the results of the three-year-long research, the following conclusions can be made: White Tamjanika showed positive agrobiological properties in the conditions of Zupa vine district. The climatic characteristics of the investigation year were very good for this cultivar. The highest number of bunches per bud was observed in the fifth and sixth variants with the highest load (1.26), and the lowest in the second variants with the lowest load (1.12). The highest number of bunches per developed cane was in 2009 (1.42), very significantly higher than in 2010 (1.36), which recorded the lowest number of bunches per developed cane. The number of bunches per productive cane is quite a variable feature, which is very dependent on the agroecological conditions, and varied from 1.40 (second variants) to 1.72 (sixth variants). With the increase in the fertile bud loads per vine increased the number of bunches per vine.

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STORAGE PROTEIN VARIATION IN CENTRAL EUROPEAN WHEAT VARIETIES

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Abstract

Genomes of wheat varieties provide an available and highly efficient polymorphic system of genetic markers. Suitable protein markers in wheat are endosperm storage proteins – gliadins and glutenins. The HMW glutenin subunits were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and gliadins by acid polyacrylamide gel electrophoresis. The objective of this study was to determine the composition of high molecular weight glutenin subunits and gliadin block of 94 European varieties of hexaploid wheat (*Triticum aestivum* L.) The allelic block *Gli-1B3*, the marker of rye translocation *1RS/1BL* as well as the marker of poor bread-making quality was detected in 27 genotypes. Sixteen high molecular weight (HMW) - glutenin subunits (GS) were found, three belonged to *Glu-1A*, nine to *Glu-1B* and four to *Glu-1D* locus. The most frequent (32, 9%) HMW-GS at the *Glu-1A*, *Glu-1B* and *Glu-1D* complex loci were 0, 7+9, and 5+10 respectively. Bread quality (class) in the examined accession varied from minimum value 4 to maximum value 10.

Keywords: *Triticum aestivum* L., glutenin, gliadin, SDS-PAGE, A-PAGE

Introduction

The quality of wheat flour for bread making depends on the viscoelastic properties of the dough, which are influenced by the quantity and quality of the gluten-forming storage proteins of the endosperm. These proteins consist of two classes, monomeric gliadins and polymeric glutenins, a classification based on the disulfide-bonding behavior of the individual proteins. The gliadins are monomeric proteins that either lack cysteine residues (omega gliadins) or have only intra-chain disulfide bonds. Glutenin subunits bind to each other forming polymers linked by disulphide bonds. After the reduction of disulfide bonds, glutenin subunits can be divided in two main groups: high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS), based on relatives mobilities in SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The qualitative composition of the HMW and LMW glutenin subunits of a variety is a genetically determined characteristic, and they are not affected by variation in environmental conditions. The HMW glutenin subunits (HMW-GS) are encoded by *Glu-1A, Glu-1B,* and *Glu-1D on* the long arm of chromosomes 1A, 1B and 1D respectively. The LMW glutenin subunits (LMW-GS) are encoded by *Glu-3A, Glu-3B,* and *Glu-3D on* the short arm of these chromosomes. The HMW-GS can be classified according to their electrophoretic mobility, their structure and composition. Molecular analyses have shown that each *Glu-1* locus contains two genes, one encoding a higher molecular weight x-type subunit, the other a lower molecular weight y-type subunit. The absence of subunits in some cases has been proved to be due to gene silencing. Most of bread wheat cultivars possess from three to five active HMW-Gs (Payne at al., 1979). Usually, the *Glu-1D* locus encodes both types, the *Glu-1B* locus encoded both types (x-type and y-type) or one x-type subunit, and the *Glu-1A* locus can have one or none active subunits. Unfortunately, it is not a simple matter to develop wheat lines that consistently express high levels of glutenin. Glutenin proteins are encoded by six genes and the total glutenin content of the grain

is proportional to the expression of these genes. However, specific gene silencing often results in one or more of these genes not being expressed, with the result that varieties may not reach optimal glutenin content. Seed storage proteins are considered to be usable markers for the studies of wheat genetic resources (Bradová et al., 2005; Gálová et al., 2002; Gregová et al., 2007; Šramková et al., 2010). From them, glutenins are in addition associated with the technological quality of wheat flour and the specific gliadin alleles are associated with plant earliness and cold resistance (Metakovsky & Branlard, 1998). Payne et al. discovered a correlation between the presence of certain HMW-GS and gluten strength, measured by the SDS-sedimentation volume test. On this basis, they designed a numeric scale to evaluate bread-making quality as a function of the described subunits *Glu -1 quality score* (Payne et al., 1983). Assuming the effect of the alleles to be additive, the bread making quality was predicted by adding the scores of the alleles present in the particular line. It was established that the allelic variation at the *Glu-1D* locus have a greater influence on bread-making quality than the variation at the other *Glu-1* loci (Payne at al., 1984). During the last few years an increasing interest of wheat breeders for genetically adapted and diverse raw material can be detected, mainly influenced by the need for quality traits, specific adaptability, and yield stability of wheat. The objective of this work was detect genetic backround for bread-making quality based on variations of HMW-GS in *Triticum aestivum* L. genetic resources originated from the Europe.

Material and methods

We analyzed seed storage proteins, which were extracted from 94 genotypes of hexaploid wheat (*Triticum aestivum* L.) grain originating from seven different geographical areas (Slovakia, Austria, Hungary, Germany, Poland, Sweden and Czech Republic) of Europe in 2014. All samples were obtained from the collection of genetic wheat resources of the Gene Bank of Slovak Republic in Piešťany. Seed storage proteins were isolated from the endosperm of intact, dry and mature single seeds. Glutenins were extracted by a standard referee method ISTA and were performed by discontinuous PAGE based on ISTA methodology (Wrigley, 1992) using the electrophoretic unit Protean II (BioRad). Standard ISTA reference method (A-PAGE) was used to extract alcohol-soluble proteins-gliadins (Draper, 1987). Protein fractions were stained by Coomassie Brilliant Blue R – 250. The separate gluten subunits were identified by the nomenclature of Payne and Lawrence (1983). Bread-making quality of the grain is expressed as a score (*Glu-score, Rye-score*) deriving from the presence or absence of specific HMW-GS and gliadins.

Results and discussion

Wheat accessions originating from Slovakia (44), Austria (3), Germany (13), Poland (6), Czech Republic (10), Hungary (15) and Sweden (3) were evaluated for high molecular weight glutenin subunits using SDS – PAGE. Each cultivar from four and five HMW subunits, where *Glu-1A* encoded one or no subunits, *Glu-1B* encoded one or two subunits, and *Glu-1D* encoded two subunits, depending on allele.

Three alleles were identified at *Glu-1A* locus subunits 1, 2* and null allele (Figure 1 and Figure 4), five alleles encoding subunits 7+8, 7+9, 17+18, 20 and 6+8 (Figure 2 and Figure 5) at *Glu-1B* locus and two alleles at *Glu-1D* locus encoding 2+12 and 5+10, respectively. (Figure 3 and Figure 6).

The alleles were found at widely differing frequencies in wheat genotypes analyzed. The most frequent allele was at *Glu-1A* null allele (64,9%), at the *Glu-1B* encoding 7+9 (50%) and at *Glu-1D* encoding 5+10 (73,4%), respectively.

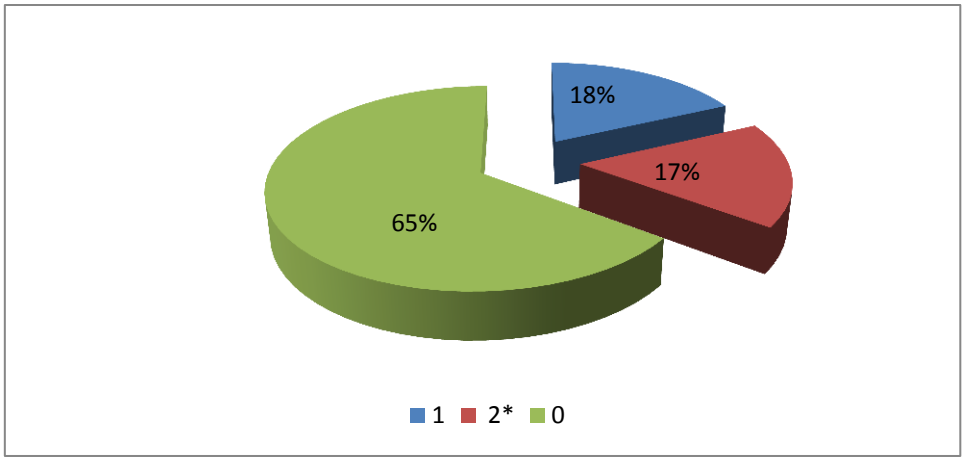

Figure 1 Allelic frequency at *Glu – 1A* locus

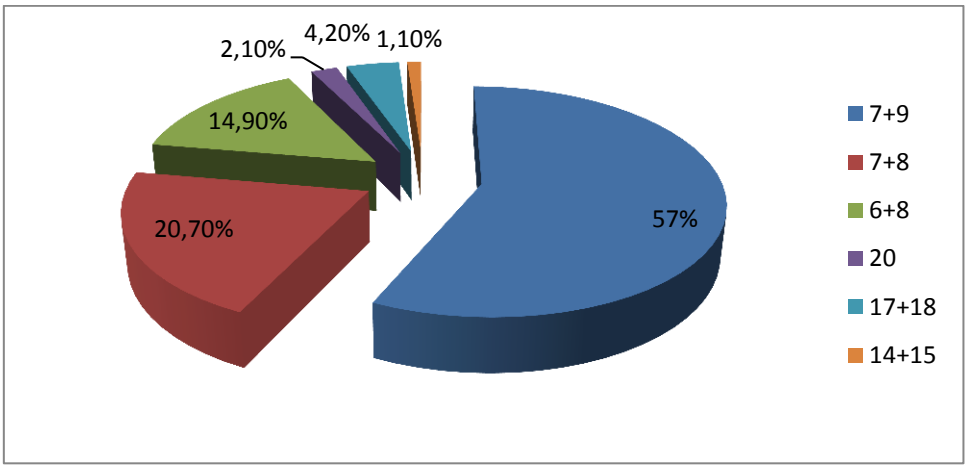

Figure 2 Allelic frequency at *Glu – 1B* locus

At the *Glu-1A* locus, subunit 1 and subunit 2* were found to be expressed in 17 and 16 cultivars, respectively, and the null subunit was present in 61 cultivars. Most cultivars (64,9%) had the null subunit, which has no relevance to superior bread making quality. Distribution of the HMW glutenin subunits revealed that advanced lines having subunit 1 or 2* encoded by *Glu-1A* locus possess better bread-making quality attributes because of the linear relationship of these fragments with higher extensibility and better dough strength.

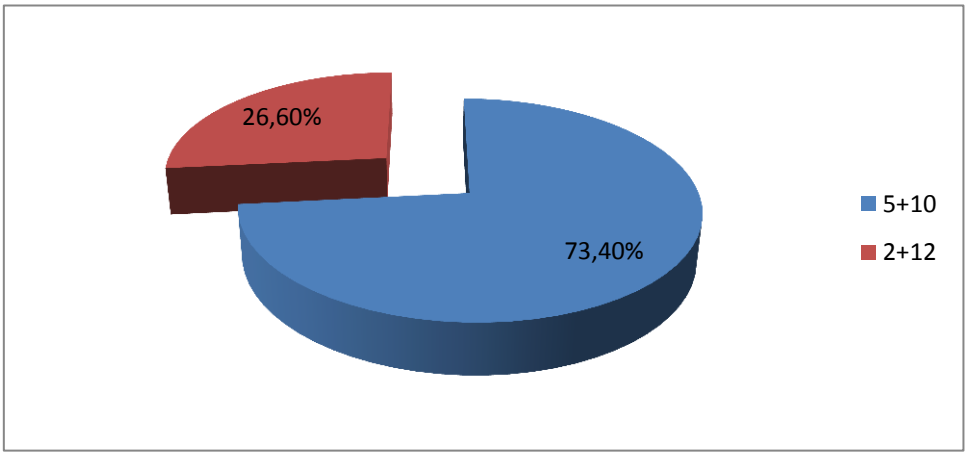

Figure 3 Allelic frequency at *Glu – 1D* locus

Six different compositions 7+9, 7+8, 6+8, 17+18, 20, 14+15 were found at the *Glu-1B* locus with subunits 7+9 predominant (54 cultivars) which according to Payne (1987) contributes to good bread-making quality. Relatively rare allele *Glu-1B* 17+18 and *Glu-1B* 14+15 associated with good bread making quality was found. In Slovakian genotype Veldava very rare subunit 20 was identified and appeared with low bread making quality. Subunits that were studied most are those encoded by the *Glu-1D* locus. Payne et al. (1979) were the first to discover that subunits 5+10 at the *Glu-1D* locus occurred in wheats with high bread-making quality and subunits 2+12 in wheats with low bread making quality. This discovery was subsequently confirmed by several authors (Gálová et al., 1998; Békés, 2011). Most of the cultivars were found to express subunits 5+10 (69 cultivars) at the locus *Glu-1D*.

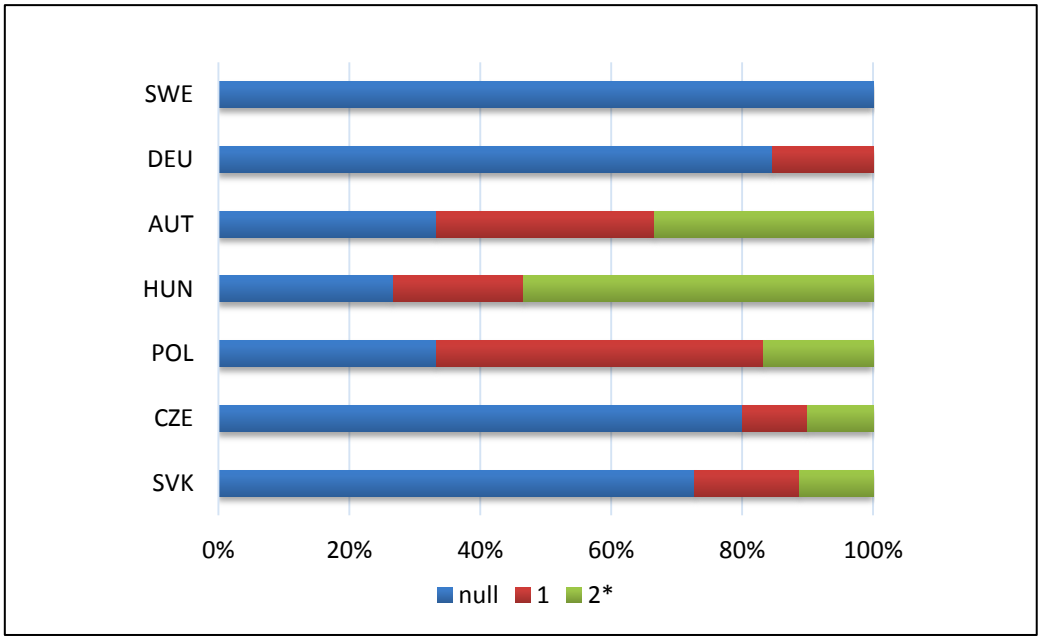

Figure 4 Allelic frequency at *Glu – 1A* locus according to the country of origin

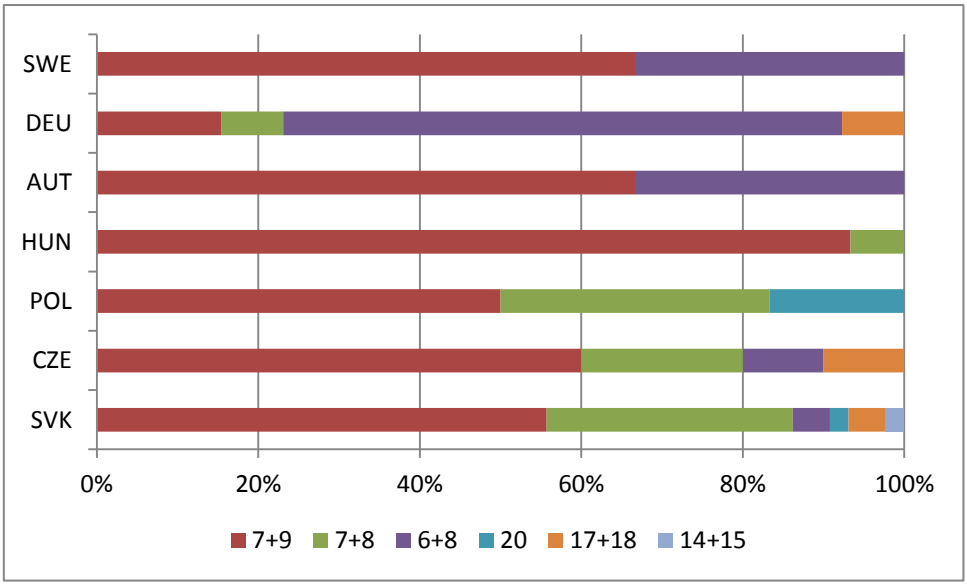

Figure 5 Allelic frequency at *Glu – 1B* locus according to the country of origin

The presence of *Gli-1B3* secalin allelic block (bread-making quality inhibitor) is a consequence of translation of rye chromosome segment into wheat genome (*1RS/1BL*). The allelic block *Gli-1B3*, the marker of rye translocation *1RS/1BL* as well as the marker of poor bread-making quality was detected in 27 genotypes. In order to predict the bread making quality of wheat genotypes, *Glu-score* was calculated for the wheat genotypes on the basis of high molecular weight glutenin subunits detected. Our data showed that the *Glu – score* in wheat genotypes varied within an interval from 4 to 10. One Polish cultivar Simbol and five Slovak genotypes (Axis, Klaudia, Viola, IS Escoria and IS Gordius) reached the highest value of *Glu–score* (10).

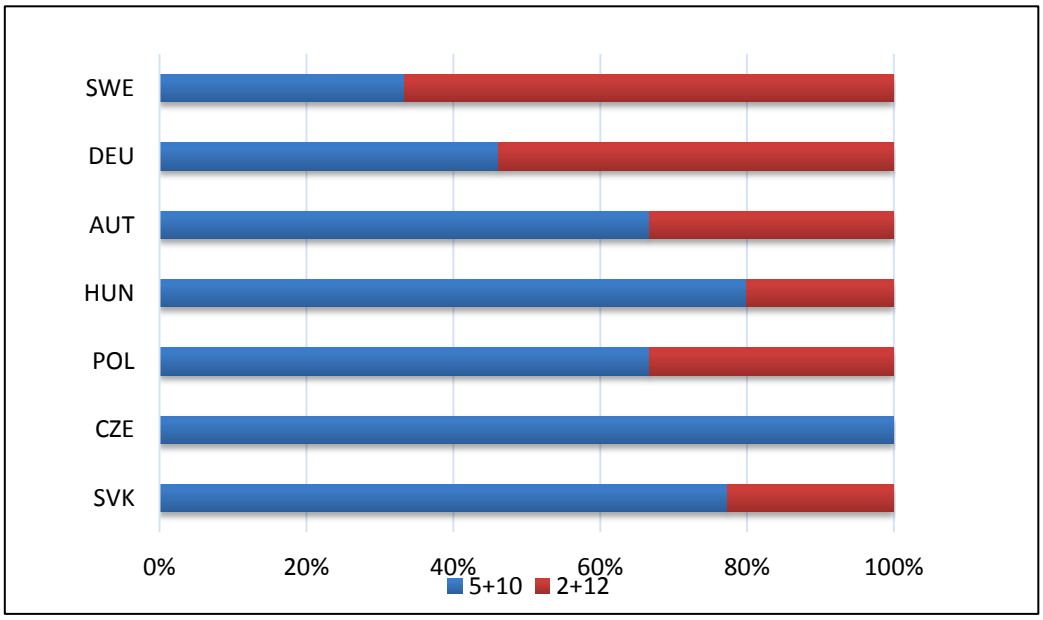

Figure 6 Allelic frequency at *Glu-1D* locus according to the country of origin

One Slovak cultivar (Veldava), one Swedish cultivar (Meridiem) and four German cultivars (Previa, Previna, Windsor and Korsika) reached the lowest value of *Glu- score* (4). Overall, Slovak, Hungarian, Czech and Polish wheat collections had a good technological quality.

Conclusion

The composition of both HMW glutenin subunits and gliadins contributed to the variation in gluten quality. Results reported herein indicate that in the case of wheat varieties the *Glu-score* can be used to predict bread making quality. Accordingly, the score can be used to screen breeding populations for bread making quality.

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DETERMINATION OF VEGETATIVE AND GENERATIVE CHARACTERISTYICS OF DIFFERENT COTTON VARIETIES UNDER DROUGHT STRESS

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Abstract

The research was carried out to determine the effect of different irrigation water requirements of different irrigation levels (mm), on the evapotranspirasyon seed yield (kg da^{-1}) with vegetative and generative properties of two cotton types (drought tolerant, BA 525) and (drought susceptible, Lydia),in the Eastern Mediterranean region (Turkey). Drought or water deficit stress elicits many different phenological responses in plants. The study of vegetative features included the wet and dry weight of plant (gr), leaf area (cm^2), number of leaves (number plant^{-1}), number of fruit branches (number plant^{-1}), number of bud formation (number plant^{-1}) and number of node (number plant^{-1}). This result indicates that water stress in plants limit the phenological development significantly and The average of all measurements made after flowering has been observed to decrease. According to this research the average value of the weakening due to stress and aging undermines vegetative features significant effect on yield.

Keyword: *Cotton, Irrigation Level, Drought, Drought tolerance, Growth Periods*

Introductıon

Drought (water stress) is one the major abiotic stress factors that affect all organisms lives including human in terms of health and food. Plant responses to drought are complex, and different mechanisms are adopted by plants when they encounter drought (Jones, 2004). These mechanisms can include: (1) drought escape by rapid development, which allows plants to finish their cycle before severe water stress; (2) drought avoidance by, for instance, increasing water uptake and reducing transpiration rate by the reduction of stomatal conductance and leaf area; (3) drought tolerance by maintaining tissue turgor during water stress via osmotic adjustment, which allows plants to maintain growth under water stress; and (4) resisting severe stress through survival mechanisms (Izanloo et al., 2008).The plants crops are imperative to improve the drought tolerance of crops under the changing circumstances.

Water stress has been identified as a factor that negatively affects the ratio of reproductive to vegetative growth, seed yield and its components (Iannucci and Martinello, 1998). When water stress is reduced from -1.0 to -2.0 MPa, cells become smaller and leaves develop less, resulting in a reduced area for photosynthesis. At these water potentials, ion transport is slowed and may also lead to a decrease in yield (Medrano et al., 2002). Achten et al. (2010) observed that drought treatment significantly influenced growth, biomass allocation, allometry and leaf area.

The objective of this study is to investigate the effect of water stress on growth,vegetative and generative characteristyics of two cotton cultivars grown in the Antakya of Turkey. A number of growth parameters were determined under water deficit stress, including plant height, leaf area, dry matter, number of leaves, number of fruit branches, number of node and total plant dry weight.

Materıal and method

In this study four recently improved varieties of ProGen Seed firm of Turkey, namely as BA525 (drought tolerant), and LYDIA (drought sensitive) were evaluated in a split plot design; 4 different irrigation levels, 3 replications and each replication consists of 15 meters. Each irrigation level is planned 4 ordinary, and intra-row spacing is 0,70 m, row spacing is 15 cm. The first irrigation is made when consumed 50% of the suitable capacity the subsequent irrigations are also completion of the missing moisture (about 6 days intervals). Irrigation issues are waterless (waterless, I_0), full irrigation issue (I_{100}- the missing moisture is brought to field capacity), 66% and 33% of full irrigation. 120 cm layer is discussed for determination of moisture content. In the irrigation, the area of land to be soaked was acknowledgment 35% because of the drip irrigation is used and long annual average rainfall in the region is more than 72 mm (Yıldırım, 2008).

Plant samples were taken to determine the effects of drought on cotton vegetative features 3rd period (first flowering, mid flowering and boll formation). Plant samples were taken in each iteration from 50cm. Properties studied in plants are **Plant Hight (cm):** Plants is measured as the growth cone cm distance from the top of the cotyledon leaves. After than the average of these values is taken. **The Number of Fruit Branch (number/plant**): Primary fruit branches were counted formed on the main stem of the plant and average of these values is taken. **Leaf Area (cm^2/plant):** All the leaves of plants were taken from the stem and plant area were measured with a LICOR Laser Area Meter. After than the average of these values is taken. **Seed Cotton Yield (kg da^{-1}):** Whether harvesting crops from each repetition, weighed 0.01 sensitive scales and averaged.Plant from vegetative features are number of leaves, number of bud, and number of node was counted on the example of plants and averaged. **The Number of Bud Formation (number/plant):** Primary bud formations were counted formed on the main stem of the plant and average of these values is taken.**The Number of Leaves (number/plant**): All the leaves of plants were taken from the stem and average of these values is taken. **The Wet and Dry Weight of Plant (gr):** samples taken from the field for biomass plants were weighed immediately by precision scales after than the average of these values is taken. After standing leaf area measured plants for 48 hours in 70^0C, dry weight was measured the average of these values is taken.

Result and dıscussıon

Irrigation Water and Evaporation: Both cotton varieties in the test were irrigated 8 times. Same amount of water was given to the kind of cottons during the irrigation period, respectively compared to I_{33}, I_{66}, I_{100} subjects; 433 mm, 852 mm, 1287 mm irrigation water was given to plants. Evaporation was determined respectively compared to I_{33}, I_{66}, I_{100} subjects in Lydia are 195.5, 532.4, 942 mm; in BA525 are 206.9, 532.4, 945.0 mm. Evapotranspiration occurred at very low levels (53mm) in I_0 subject because of that it was not rain.

Yield: Drought resistant (BA525) and drought-sensitive (Lydia) the difference between the yields of the cultivars were significant at $p < 0.001$ level (Table 1). When the type of average value calculated BA525 type 490 767 kg / da, Lydia type 377 150 kg / da was produced. 23,15% is calculated as the difference in yield between varieties. When type of efficiencies realized in different irrigation levels were assessed, at I_0 average 187.80 kg / da, at I_{33} 391.08 kg / da, at I_{66} 541 567 kg / da, at I_{100} 615 383 kg / da yield was obtained. Average yields took place in a different group at each level of irrigation.

Table 1. Analysis of variance results for the yields

Variance Source	Sd	Sum of Squares	F
Variety	1	77452.482	45.359***
Irrigation Level (Sd)	3	641560.648	125.239***
Variety * Sd	3	15638.628	3.053od
Error	23	761972.738	

The response to both types of irrigation levels have varied considerably. For the BA525 on I_0 varieties 239.9 kg / da., on I_{33} 415.17 kg / da., on I_{66} 596.9 kg / da. and on I_{100} 711.0 kg /da; In Lydia varieties at I_0 135.7 kg / da., at I_{33} 367.0 kg / da., at I_{66} 486.2 kg / da. at I_{100} 519.7 kg /da are also measured. On the difference between watering levels of type has been calculated as at I_0 43%, at I_{33} 11.6%, at I_{66} 18.5%, at I_{100} 27%. As we have seen, the biggest difference in yield occurred thirsty housing. This suggests that an extremely powerful mechanism of drought resistance varieties of BA525. Author (2009), the yield decrease in soil water deficit degree stated that stress sensitivity of plants and effective atmospheric conditions.

Vegetative and Generative Results: Variance analysis of the number of leaves, average of leaf area, plant height, the number of fruit branches, number of the bud formation, number of nodeand and wet and dry weight of plant forming the material value of the range results and the groups are formed according to DUNCAN test are given in Table 2,3,4,5.

The number of leaves, average of leaf area, plant height, the number of fruit branches, number of the bud formation, number of nodeand in the resistant varieties (BA525) were found higher than in susceptible varieties (LYDIA) in all of the irrigation levels (Figure 1). In this research, average leaves area of plants are measured I_0=564.056, I_{33}=865.056, I_{66}=1701.44, I_{100}=10576.278 cm^2. Average leaf areas were showed diversity between varieties ($p<0.05$). The average leaf area was found insignificant for variety and variety*irrigation levels interaction. Increased levels of irrigation increased leaf area. Birda et all. (1998) reported that number of leaves per plant, increases the leaf area index.

The plant height was varied depending on the variaties ($p>0.05$), irrigation levels ($p<0.01$), and variaties*irrigation levels of interactions ($p>0.05$). Plant height, number of leaves and leaf area occurred about quietest waterless (I_0). Irrigation level increased plant height, leaf number and leaf area was also increased (Figure 1). Asraf and Iram (2005) reported that, limiting the development of plant water stress one of the most important factors. Sankar et al. (2008) in a study conducted in five different varieties of okra found that drought stress causes leaf area and net assimilation rate decreased compared to control plants.

When the average number of fruit branches examined in terms of the cultivars, the number of the average fruit branches of BA525 is number of 6,472 and at the LYDIA is also 5,639. The number of fruit branches have varied according to the level of irrigation ($p<0.01$), varieties ($p>0.05$) and variety*irrigation level of interactions was found insignificant (Table 4).The maximum number of fruit branches were observed in BA525 (7,889). In both kinds of fruits branches also increased by increased irrigation levels. Mert (2005) showed that different reactions of genotypes in the growth period in order to determine the impact of irrigation on different genotypes study which was conducted in Hatay conditions. The number of fruits branches, plant height, number of cocoons in all varieties of decrease at the application of irrigation is not done.

Plant wet weight (stem+leaves) varied depending on the varieties ($p>0.05$), irrigation levels ($p<0.01$), and varieties*irrigation levels of interactions was found insignificant. Plant wet weight was realized with the lowest dry matter (I_0) and average of the wet weight of the I_0 is 444,167 gr. Irrigation levels increases plant wet weight increased. Plant wet weight of the two varieties of waterless condition occurred at about the same level (BA525 444,111gr and LYDIA 444,222gr). Irrigation levels has created two different groups in terms of wet weight

of plants. I_{33}, I_{66}, I_{100} were included in the same group. Drought stress occurred as a result of cell dehydration, plasma membrane resulting sag and released hydrolytic enzymes causes cytoplasm autolysis, eventuall The slowdown in growth and turgor reduction occurs (Kalefetoğlu and Ekmekçi 2005).

In our research, the dry weight (stem+leaves) of the plants are created differences between varieties but it was found insignificant and irrigation levels (*p>0.05).*In Duncan group, irrigation levels are divided into two groups. I_{100} and I_{66}, I_{33} and I_0 are in the same group. Average dry weight of the BA525 is measured that 128,417gr, in LYDIA 123,639gr also. maximum average of dry weight is calculated in BA525 variety. Tsuji et al. (2003) in study of sorghum, and Alexieva et al. (2001) in study of wheat, they are emphasized that drought stres results wet and dry weights of losses of plants.

Water stress has a major impact on the production of mainstem nodes and thus the number of fruiting branches and fruiting sites (Eric 2011). In this research the number of node and bud formation were increased by irrigation level increased (Figure 1). The number of nodes and bud formation showed differences between varieties but it was not important level (*p>0.05*) (Table 2,3). The average number of nodes in both varieties were found about the same (BA525=10.278 and LYDIA=10.028). The average number of bud formation disparity between varieties were calculated that I_0=3.0-2.4 (18,66%), I_{33}=3.78-3.44 (8,9%), I_{66}=5.0-4.22 (15,6%) and I_{100}=5.11-5.0 (2,15%). The average number of node and bud formation were affected *p<0.01* of level from the irrigation levels.

Table 3, Variance results related to number of node

Variations Supply	Sd	Sum of Square	F
Variety	1	3,556	1,575od
Irrigation level (Sd)	3	58,889	8,697**
Variety * Sd	3	1,111	0,164od
Error	64	144,444	

Sd: Degree of freedom

Table 2. Variance results related to number of bud formation

Variations Supply	Sd	Sum of Square	F
Variety	1	1,125	0,893od
Irrigation level (Sd)	3	641,375	169,620**
Variety * Sd	3	0,123	0,040od
Error	64	80,667	

Table 5. Variance results related to leaf area

Variations Supply	Sd	Sum of Square	F
Variety	1	12,500	7,392*
Irrigation level (Sd)	3	81,000	15,967**
Variety * Sd	3	6,056	1,194od
Error	64	108,222	

Sd: Degree of freedom

Table 4. Variance results related to number of fruit branch

Variations Supply	Sd	Sum of Square	F
Variety	1	1347808,347	5,894*
Irrigation level (Sd)	3	1239295042,931	1806,44**
Variety * Sd	3	922454,486	1,345od
Error	64	14635577,111	

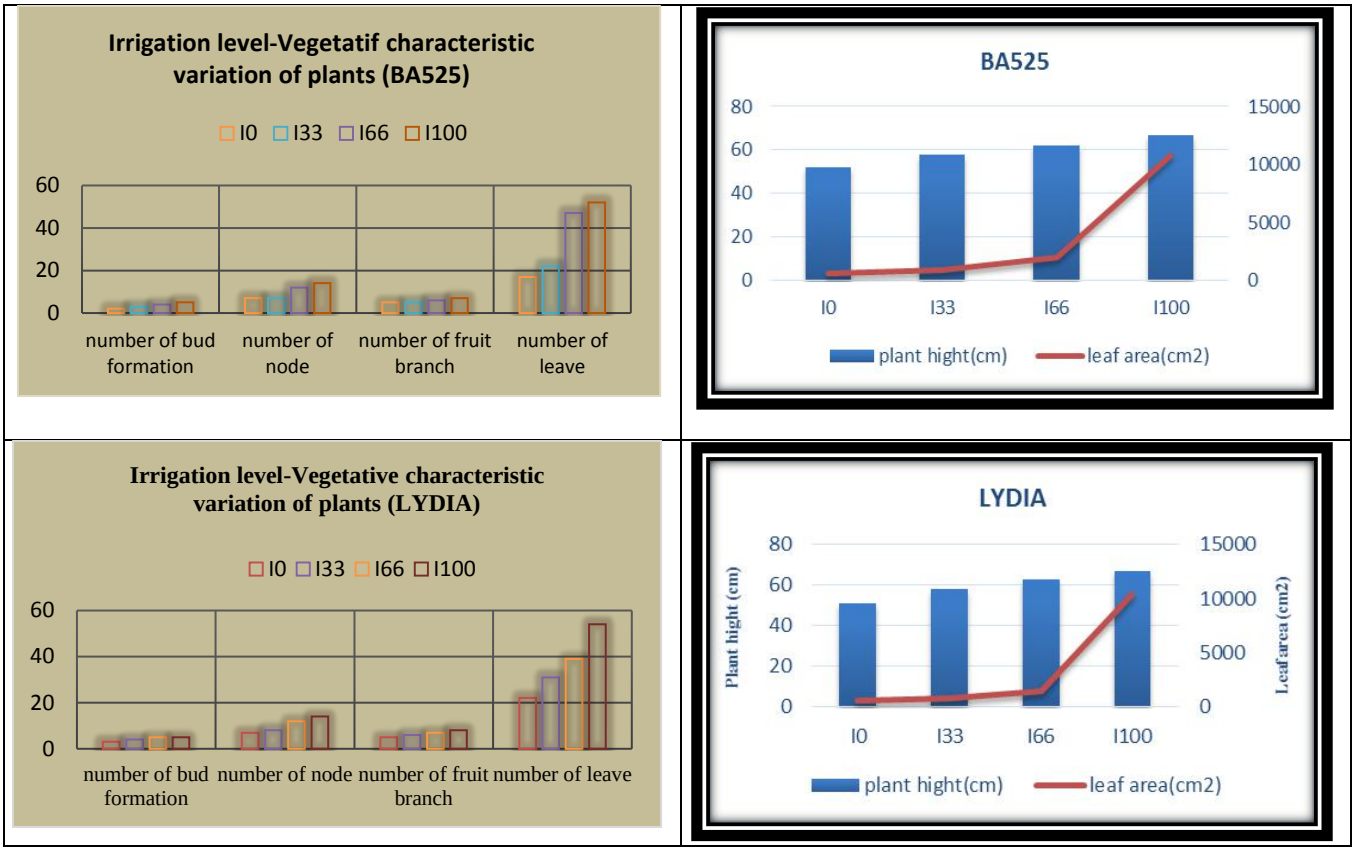

Figure 1. Change of the vegatitive measurement depending on the level of irrigation and varieties

Results

Water availability is potentially one of the most limiting factors to profitable cotton (*Gossipium hirsutum* L.) production.This study aimed to identify which parameter is more reliable in terms of vegetative and generative for determining the response to water stress of two distinct cotton varieties.

According to the analyzed characteristics, also it showed that in both variaties are significantly affected by the water stress. Average number of bud formation, node, leaves, plant height, plant wet weight and dry weight properties are unaffective significantly from variaties but they are affacted significantly from irrigation levels. Average number of fruit branch and leaf area are affected significantly from irrigation levels and variaties. To estimate the efficiency of determining the characteristics of the period examined developments (bud formation and flowering) constitute the yield of cotton is expected to be more effective. Because of that the average value of the weakening due to stress and aging undermines vegetative features significant effect on yield.

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EFFECT OF DIFFERENT ZINC APPLICATIONS ON YIELD, YIELD COMPONENTS AND SOME QUALITY TRAITS OF BREAD AND DURUM WHEAT VARIETIES

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Abstract

This research was conducted at the Research farm of the Faculty of Agriculture, University of Süleyman Demirel (Isparta, Turkey) during a period of two years (2009-2011)to determine effects of different zinc application on yield and some agronomic characters of bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.). The study was carried out on 4 wheat genotypes (bread wheat: Altay 200, Gün 91; durum wheat: Kızıltan 91, Kunduru 1149) on split-plot design in a Randomized Complete Block with 3 replications. The cultivars took place in the main plots whereas the zinc application doses (0, 9, 18, 27 and 36 kg ha^{-1} Zn) were in the sub-plots. Sowing was done by plot sowing machine providing 500 seeds per m^2. Phosphorus and nitrogenous fertilizers providing 40kg $ha^{-1}P_2O_5$ and 80kg $ha^{-1}N$ were appliedin the plots. According to the results including two years averages, zinc application had significant effect on properties examined and effect of wheat varieties had varying effects. Zinc application had positive effect on plant height, number of spike per square meter, biological yield, grain yield, harvest index, thousand kernel weights, crude protein content and zinc content in grain. The highest doses of zinc application negatively affected the examined agricultural properties and statistically, the mean values of studied trait decreased significantly.

Key words: Bread and durum wheat varieties, grain yield, yield components, quality

Introduction

The product group which has the maximum cultivation and production in the world is cereals. Cereals are widely used in animal feed and industry as well as human nutrition. Nearly 60% of the daily calorie intake is provided by wheat in less developed and developing countries (Çakmak et al., 2004). Also in these countries, where cereal-based food dominant in the diet, Zn deficiency is expanding rapidly with increasing number of micronutrient deficient people (Çakmak et al., 1996). Any increase in micronutrient concentrations in wheat seeds, has a significant impact on reducing micronutrient deficiencies.

Although the average yield in Turkey has increased to 300 kg/da in recent years, it is less than the yield obtained in developed countries. Therefore, it is necessary to improve the yield per unit area of wheat. In addition to other measures, this is only possible with the adequately balanced nutrition of plants. In our country's agricultural land, due to the use of fertilizer containing potassium, nitrogen and phosphorus for years, washing losses, and a little amount of green manure and farmyard manure usage. The balance of plant nutrients in our soil deteriorates and their benefits to the plants decrease. As a result, the area under cultivation in our country, the lack of micro-nutrients such as Fe, Mn, Cu and Zn (zinc) which are important in terms of plants has emerged. Due to different reasons, the plant grown in the agricultural lands, where plant nutrients are unbalanced, the deficiency of micro nutrients such as Fe, Mn, Cu and Zn occurs. There is zinc deficiency in about half of the agricultural land in Turkey (49.8%). In particular, this deficiency has been reported to be higher in Central Anatolia (Çakmak et al., 1996).

Conveniently zinc content in the soil of Isparta was determined Isparta 0.10 ppm (Özgüven and Katkat, 2002). On the other hand, 0.5 mg kg^{-1}andless ofthe zinc containing of soils is classified as weak soils (Lindsay and Norvell, 1978). Çakmak et al., (1996) have been reported to be 0.5mg $kg^{-1}$of Zn critical concentration limit in wheat fields of Central Anatolia. According to these data, it may be mentioned to be of zinc deficiency in the agricultural land of Isparta.

In this manuscript we presented that the effect of grain yield, yield components and quality of different Zn application has been studied in bread and durum wheat cultivars.

Materials and Methods

The field experiments were carried out at the Experimental Area of the Faculty of Agriculture, Süleyman Demirel University, Isparta (37° 45' N; 30° 33' E; and 1050 m), Turkey, in the 2009 and 2011 crop season under rainfed conditions. In the field experiment, two bread wheat cultivars (Gün-91 and Altay-2000) and two durum wheat cultivars (Kızıltan-91 and Kunduru-1149). The varieties are cultivated predominantly in this region by farmers. The experiment included five zinc application rates (0, 9, 18, 27 and 36 kg ha^{-1}Zn). The study was carried out on 4 wheat cultivars on split-plot design in a Randomized Complete Block with 3 replications. The varieties were placed in main plots whereas zinc application rates were placed in sub-plots. Sowing was done by plot sowing machine providing 500 seeds per m^2. The basal fertilizer applications were 40 kg ha^{-1} P_2O_5 and 80 kg ha^{-1} N as triple superphosphate and ammonium sulphate, respectively. Zinc sulphate was used as zinc fertilizer.

All of the phosphorus fertilizers were applied with sowing. Total nitrogen fertilization was applied in two equal doses, during sowing in autumn (October) and in early spring (March) when plants were at the tillering stage. Plot sizes were 9.6 m^2 (1.2 m x 8 m), with 6 rows and a row spacing of 20 cm.

Plant height was determined using 10 plants selected randomly in each plot. Thousand kernel weight was determined by weighing 100 grains three times, then multiplying their average by 10.Concentrations of Zn in grain were determined by atomic absorption spectrometry after ashing samples at 550°C and dissolving ash in 3.3% HC1 (Çakmak et al., 1996). The protein content of the samples was calculated by multiplying the Kjeldahl N by 6.25

All the above ground biomass washarvested at the maturity stage and dried in the sun (3days). The biomass was weighed for biological yield and spikes were separated from the vegetative organs. Biological yield and grain yield perhectare were calculated.

The soil of the research areas had a low water-holding capacity, was alkaline (pH 7.9-8.3) and Lime (25.5-30 % CaCO3), and contained a low amount of organic matter (1.15-1.34%). The P_2O_5, N and Zn contents were 192-197 mg/kg, 0.4-0.17 N % and 0.52-0.79 ppm, respectively. The long-term (1975-2007) total rainfall and average temperature of the location were 289.7 mm and 8.4°C, respectively.

The weather conditions during the two years were different. The long-term average temperature from September to June was 9.5 °C. Precipitation was 453.4 mm for the same period. The growing seasons (from October to June) in 2009-010 and 2010-11 had average temperatures of 10.7 and 9.8°C, total precipitation of 620.2and 459.3mm respectively. During the 2010-2011 growing season, there was quite a lot of rainfall during germination. In contrast, the 2009-2010 seasons were hotter during the seed filling stage.

Statistical analysis of the data obtained was carried out using MSTAT-c statistical package program. The calculated mean values were compared using Duncan’s multiple range Test.

Result and discussion

Plant Height

According to the averages of two years' results, compared to the control treatment, Zn application doses significantly increased plant height in the wheat genotypes (($p< 0.01$; Fig 1). However, the highest Zn application (36 kg ha^{-1}) reduced plant height. Because the response to Zn doses of wheat cultivars was different, cultivars x doses interaction was found significant. Even if Zn application in Kızıltan-91 cultivar had increased plant height, it would have been insignificant statistically (Fig. 1). As a result, plant height in all cultivars increased to a certain dose with Zn application and the highest doses reduced plant height (18 kg ha^{-1} and over these doses). Different results have been found in the studies on this subject. Whereas Bağcı (2000) showed that Zn application increased plant height in different cereals depending on the dose increase (0, 0.7 and 1.4 kg/da), Doğan et al., (2002) found Zn application insignificant in wheat.

On the other hand the toxic effect of Zn application was determined on plants. It was presented that higher Zn concentrations in the plant reduced cell division (Bobak, 1985), root height and chlorophyll amount (Bekiaroğlu and Karataglis, 2002) and made plant habitus small (Khurana and Chatterjee, 2001). In this study, it was thought that high Zn concentration in both bread and durum wheat cultivars reduced cell division, and had a negative effect on plant growth.

Number of Spike per M^2

According to the averages of two years' results, the effects of Zn doses and varieties were significant on the number of spike per m^2 ($p< 0.01$; Fig. 1). According to varieties on the number of spike per m^2 changed between 442.1 and 547.9numbers and the highest value in Kızıltan-91, the lowest one was in Altay-2000. Zn application (up to 18 kg ha^{-1}) increased number of spike per m^2. However, high Zn doses reduced number of spike per m^2. Because the reaction of wheat cultivars was different, Zn dose x cultivar interaction was found significant. Whereas the most number of spikes per m^2 in Gün-91 was 9 kg ha^{-1} Zn, it was 18 kg ha^{-1} Zn in [1]Altay-2000, Kızıltan-91 and Kunduru-1149 (Fig. 1). However, in these varieties, the differences between 9 kg $ha^{-1}$and 18 kg ha^{-1} Zn applications were insignificant. In the research Zn applications increased number of spike per m^2 in both bread and durum wheat cultivars. Similar finding was also reported by Bağcı (2000), Sönmez and Kıral (2002). Rengel and Graham (1995) showed that Zn fertilizer under Zn deficient conditions improved seedling vigor, resistance to pathogens and yield capacity of plants.

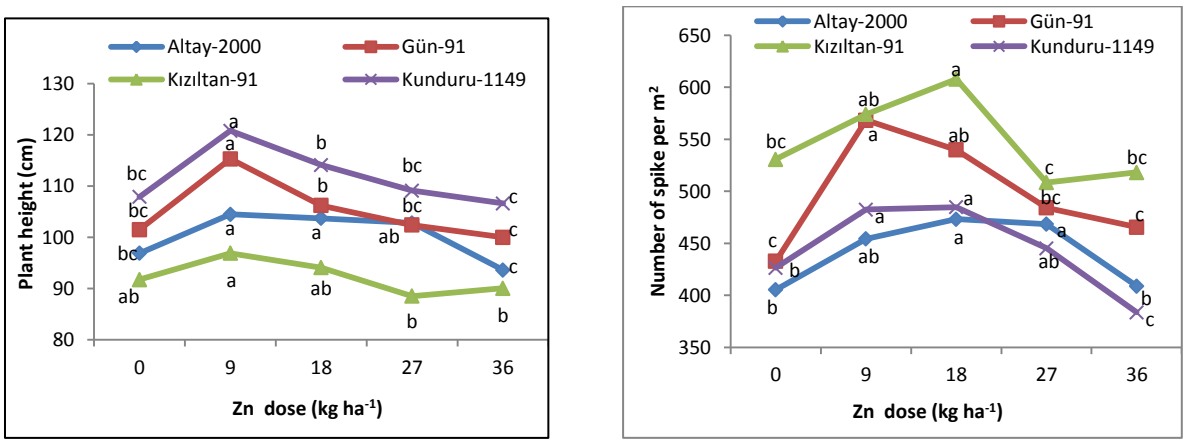

Figure 1.Effect of different zinc doses on plant height and number of spike per $m^2$of bread and durum wheat cultivars

Biological Yield

According to cultivars, two years mean biological yield changed between 10740 and 12516 kg ha^{-1} and this difference among cultivars was significant ($p<0.01$). While the highest biological yield was in Gün 91, the lowest biological yield was in Altay-2000. In general Zn application increased biological yield in cultivars and it was statically significant ($p<0.01$). Although according to control biological yield in Altay-2000 and Kızıltan-91 was 30% and it was 23% in Kunduru-1149, Zn applications were insignificant in Gün-91. For that reason cultivar x dose interaction was significant ($p<0.01$).

On the other hand, the highest concentrations of Zn applications reduced biological yield. In the research except for Gün-91, in the other cultivars Zn application increased biological yield up to 18 kg ha^{-1} (Fig. 2), but the values between 9 kg ha^{-1} and 18 kg ha^{-1} were statically in the same group.

Similar finding was also reported by Irmak (2002), and Özcan (2004) that the response of genotypes to Zn applications was different. Eker et al., (1999) showed that the response of genotypes to Zn deficiency could be different and the resistant varieties as to the sensitive ones could take more Zn from the soil. It was reported that Zn in plant tissues could also be used in different efficiencies (Torun et al., 1998). Otherwise high consentration has affected plant growth negatively (Khurana and Chatterjee, 2001; Bekiaroğlu and Karataglis, 2002).

Grain Yield

Variety, Zn applications and their interactions on grain yield in bread and durum wheat varieties were statistically found significant ($p< 0.01$).Different Zn applications in wheat cultivars had a positive effect on grain yield. According to two years mean the highest grain yield was in Gün 91 (3768 kg ha^{-1}), the lowest grain yield was in Kunduru-1149 (3229 kg ha^{-1}) (Fig. 2). The effect of Zn applications on varieties was different and while according to control (0 kg Zn ha^{-1}) the increase ratio in grain yield in Altay-2000 was 51.62%, this ratio in Gün-91 was 15.84%. But the increase rate in grain yield of durum wheat cultivars was similar to each other (Kızıltan-91, 38%; Kunduru-1149, 36%). Relativeincreases in grain yield of wheat by soil application of Zn in the Central Anatolia region varied between 5% and 554% with a mean of 43 %(Cakmak et al., 1996). Except for Altay-2000, in other varieties, grain yield obtained from Zn sulphate application between 9 kg $ha^{-1}$and 18 kg ha^{-1} was found insignificant. As to these results, sensitivity of varieties to Zn deficiency was different. The response of varieties to Zn deficiency could be related with the ability of taking Zn from the soil and using in different efficiency in plant tissues (Torun et al., 1998). On the other hand, it was reported that high Zn concentration reduced plant growth, seed number and seed weight (Khurana and Chatterjee, 2001). Thus in this study less grain yield in high concentration (36 kg ha^{-1}) showed the toxic effect of Zn.

In agreement with the previous reports (Yılmaz et al., 1997; Kalaycı et al., 1999), it was determined to be different response of zinc deficiency in wheat varieties. Zn efficiency for grain yield has been reported to range between57-92% (Kalaycı et al., 1999). Other than this,the increases in grain yield by Zn application were higher than those on biomass (Fig. 2). These results indicate that the importance of Zn nutrition for seed set is greater than for vegetative growth.

In this study, the grain yield of without applying Zn plants was significantly lower. Among the reasons, these are showed, less number of spike per m^2 and the weaker of the plant development (Fig. 1 and 2; Çakmak et al., 1989).Brown et al. (1993) reported that anther and pollen grain development in wheat plants having Zn-deficient were largely impaired, possibly as a result of reduced levels of indole acetic acid and proteins. In addition to, Sharma et al., (1979) reported that under Zn deficient conditions, there was increased abscisic acid concentration and inhibition of anther and pollen grain development in flowers.

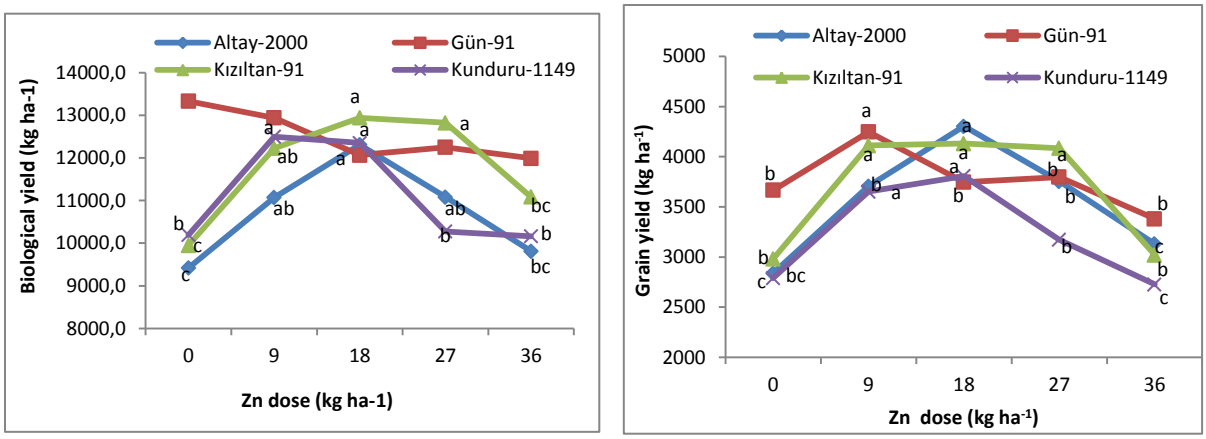

Figure 2.Effect of different zinc doses on biological yield and grain yield of bread and durum wheat cultivars

Harvest Index

According to the average of two-year results, Zn applications in wheat varieties on harvest index had a positive effect and it was statically found significant ($p<0.01$). In general, in all varieties the harvest index values between 9 and 27 kg ha^{-1}Zn application were statically in the same group. However the harvest index in the highest dose (36 kg ha^{-1}) reduced and this became significant (Fig. 3). In the research as two-year mean the highest harvest index was in Altay-2000 (32.25%), the lowest was in Kunduru-1149 (29.02%). The harvest index of Gün-91 (30.16%) and Kızıltan-91 (30.77%) was similar to each other. (Fig. 3). In this research, as in the other characteristics, the harvest index was affected from Zn application positively. But in the experiment the harvest index values had changes depending on the genotypes. Bağcı (2000) reported that, Zn application increased harvest index in cereals, it was 22.7-33.4% under dry conditions, 26.0-34.6% was under irrigations and the response of genotypes were different. On the other hand, it was reported that the effect of Zn application on harvest index in some researches was insignificant, and even it was less in some varieties (Doğan et al., 2002; Özcan, 2004).

Thousand Kernel Weight

According to the average of two-year results, Zn applications in wheat varieties on thousand kernel weight were statically insignificant. But the varieties and their interactions were statically significant ($p<0.01$). While thousand kernel weight in Zn doses was insignificant in Altay-2000 (38.13-40.14 g), Gün-91 (32.93-35.43 g) and Kızıltan-91 (47.06-50.43 g) it was statistically significant in Kunduru-1149. In general, thousand kernel weight of durum wheat was more than the one in bread wheat. Otherwise, thousand kernel weight was reduced in durum varieties by Zn application (Fig. 3). The reason of it was thought that there was a relationship with plant number in per area and grain numbers in spike. Because plant density increased depending on Zn application and it caused to reduce in grain size of spike. In agreement with the previous reports, Bağcı (2000) and Doğan et al., (2002) reported that thousand kernel weight increased by Zn application, and it was insignificant.

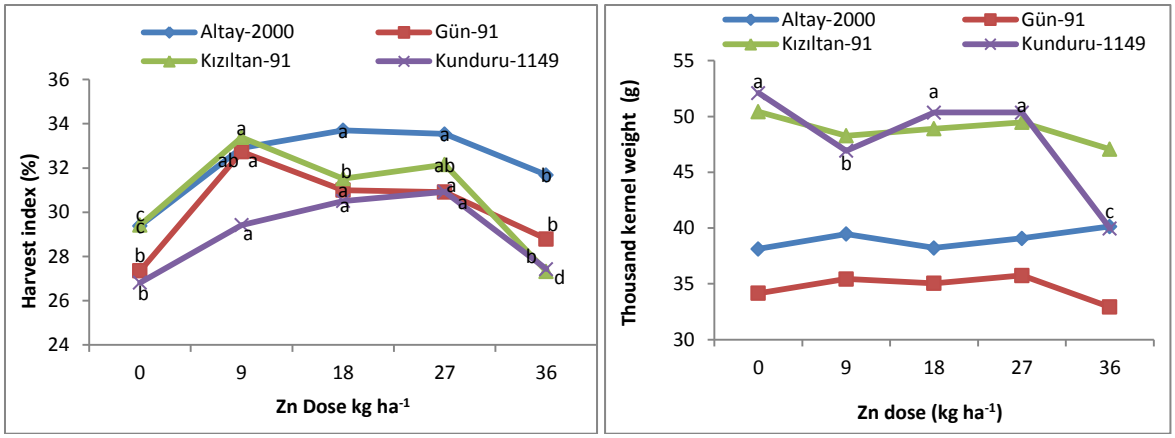

Figure 3.Effect of different zinc doses on harvest index and thousand kernel weight of bread and durum wheat cultivars

Crude Protein and Zinc (Zn) Contents in Grain

According to variance analysis, genotypes, Zn applications and their interactions on crude protein content and zinc content in grain were statistically found significant ($p< 0.01$).

The highest crude protein content obtained 27 kg Zn ha^{-1}(14.66%), the differences among other applications were significant. The lowest crude protein content (11.72%) was determined in plants not applied Zn, but the values between the highest Zn dose (36 kg ha^{-1}; 11.96%) and the lowest one were insignificant. Average crude protein content in grain in wheat varieties changed between 12.27% and13.51% and crude protein rate was found higher in durum cultivars. In the research, if response of wheat genotypes had been different by Zn application, it would have increased crude protein content (Fig.4). Generally, high dose Zn application in all varieties had an effect on crude protein content negatively. In the research mean Zn content obtained the most inKunduru-1149 (19.75 mg kg^{-1}). The Zn content was 15.02 mg kg^{-1}in Gün-91, 15.63 mg kg^{-1}in Kızıltan and 17.60 mg kg^{-1} in Altay-2000. Although response of genotypes was different by Zn applications, Zn content increased in all varieties. But the highest Zn dose reduced Zn content in grain (Fig. 4). Zn content in grain changed between 14.41 and 19.21 mg kg^{-1}depending on Zn application. The highest Zn content obtained from 9 kg ha^{-1} Zn application. The lowest one was from plants not applied Zn.

Protein content is an important criterion in determining of wheat quality. In several other studies a positive correlation between seed protein and seed Zn was found **(**Çakmak et al., 2004; Distelfeld et al., 2007). On the other hand Bağcı, (2000) reported that protein content in grain reduced by Zn applications and the negative effect under irrigation conditions was more than under dry conditions. In addition Irmak (2002) showed that the effect of Zn application on protein content was insignificant.

Several authors have reported the genotypic variation for Zn in cereals (Peterson et al., 1986; Yılmaz et al., 1997, Peleg, et al., 2008). In our study, the genetic variation for grain Zn content within bread and durum wheat varieties is observed. Furthermore, genotype x environment interaction is high and factors such as soil properties, water availability and fertilizer management have much greater effect on the micronutrient concentration than the genetic factors (Peterson et al., 1986; Çakmak et al., 2004).In our studies, average Zn content in grain by Zn applications varied from 14 to 19 mg kg^{-1}.

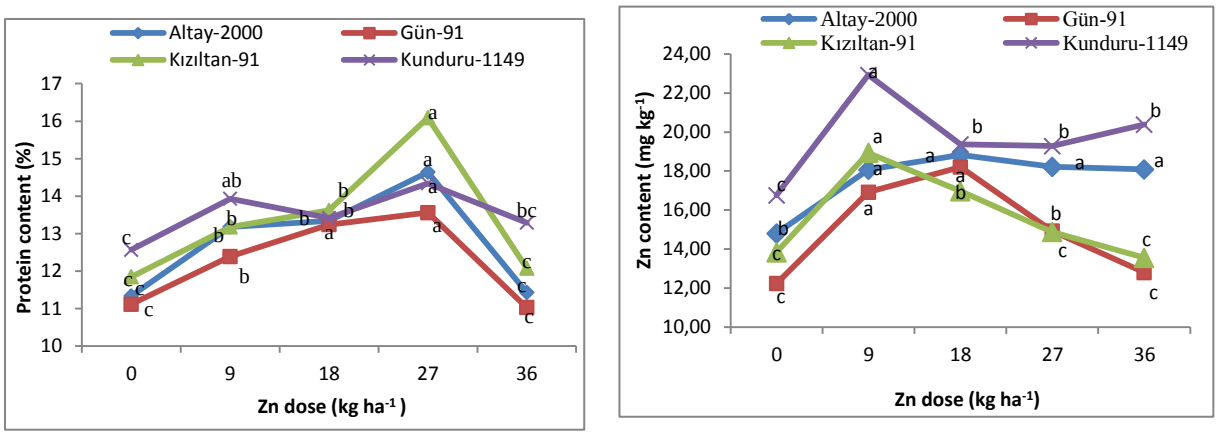

Figure 4. Effect of different zinc doses on crude protein and Zn contents of bread and durum wheat cultivars

Conclusion

According to two-year research results, Zn application in both durum and bread wheat increased grain yield and grain quality. For that reason, the application of fertilizers contented Zn could be suggested in Zn deficiency fields under Isparta conditions. For similar ecological conditions, Zn doses between 9 kg ha^{-1}and 18 kg ha^{-1}could be recommended depending on varieties.

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ORNAMENTAL PEPPER BREEDING PROGRAM AS POTTED PLANT IN TURKEY

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Abstract

Turkey is a big producer for pepper production, but it hasn't got any ornamental pepper cultivar for pot and outdoor ornamental plants. This work describes the characterization of 32 pepper genotypes (*Capsicum* spp.) considering traits related to ornamental purposes, analyzes of the morphologic characterization. Qualitative and quantitative traits were used in the program. The experiment was carried out in unheated greenhouse. Fruit length and color were the most important traits to discriminate the genotypes. Desirable traits for ornamental use as small height, intermediate growth habit, three to five stages for fruit ripening maturation, early flowering and fruit set were identified in sixteen genotypes (MKU 35, MKU 43, MKU 44, MKU 68, MKU 73, MKU 74, MKU 76, MKU 79, MKU 82, MKU 83, MKU 84, MKU 85, MKU 87, MKU 97, MKU 101 and MKU 115). Other ten genotypes (MKU 18, MKU 19, MKU 30, MKU 71, MKU 72, MKU 86, MKU 92, MKU 96, MKU 104 and MKU 106) had also ornamental interest for garden growing because of the plant height, along with fruit color, size and shape. Genotypes MKU 19, MKU 35, MKU 43, MKU 83, MKU 86, MKU 92, and MKU 96 can be recommended for crosses seeking pepper ornamental breeding, based on genetic divergence and suitable agronomic traits. Also MKU 67, MKU 69 and MKU 71 can be used for sweet (non pungent) genetic material.

Keywords: *Capsicum species, selection, hybridization, morphological characterization*

Introduction

The pepper (*Capsicum spp*.) has been used for feed of human since 7000 BC and has been cultivated since 5000 BC in America (Bosland and Votova, 1999). Even though Capsicum did not originate in Turkey, where its greatest diversity can be found, it has been cultivated and consumed there for an estimated 300-400 years and thus it is reasonable to consider it a traditional local food (Mavi et al., 2013).

Within the genus Capsicum, several cultivars possess an aesthetic value for ornamental purposes in the garden and as indoor pot plants. Ornamental peppers can provide a range of pod shapes and colors complemented by varying degrees of green or purple foliage. Classification of ornamental peppers includes cultivars within all cultivated species: *Capsicum annuum* L., *Capsicum chinense* Jacq., *C. frutescens, C. pubescens* and *Capsicum baccatum* var. *pendulum* Willd. The genus Capsicum comprises economically important species around the world, among them the most cultivated is *Capsicum annuum* L., with great diversity of forms and colors of their leaves, flowers and fruits, which gives them great ornamental potential (Stommel and Bosland, 2006).

Turkey is a big producer for pepper production, but it hasn't got any ornamental pepper cultivar for pot and outdoor ornamental plants (Mavi, 2013). There are few commercial pepper varieties headed for landscaping purposes, although national germplasm bank for Capsicum genus holds in their collections, sample that might be used genetic improvement with the objective to develop new ornamental pepper cultivars. The current research had as objective to evaluate the genetic variability aiming to assess the ornamental potential of pepper samples through selection indexes. his study aimed to characterize 32 *Capsicum* spp.

genotypes based on qualitative and quantitative descriptors related to the potted plant ornamental potential; to estimate the dissimilarity between accessions and the relative contribution of the evaluated characters and to indicate potential for the use in hybridizations to develop a breeding program for potted ornamental purposes.

Materials and methods

Thirty-two *Capsicum* spp. genotypes from the germplasm collection of the University of Mustafa Kemal, Faculty of Agriculture, Department of Horticulture (Table 1) were studied. The experiment was conducted in a greenhouse in Antakya, Hatay, Turkey, from September 2010 to May 2015. Sowing was performed in polystyrene trays filled with peat moss. The seedlings were transplanted to three-liter plastic pots containing soil, sand and manure at the proportion 1:1:1. The study used randomized block design with three replicates, with four plants per plot.

Descriptors proposed by IPGRI (1995) were used for thirty-two genotypes, nine qualitative and eight quantitative ones, observing the vegetative and reproductive traits of plants. For the qualitative descriptors related to the vegetative part, data were obtained from the mode of observations taken in three plants per accession in each block and for the characterization of the reproductive part, five fruits per plant were considered. Pungency analysis was based on the staining of the placenta. Quantitative characteristics were based on the mean values observed in each treatment in which the following items were evaluated: 1) plant height (HGT, cm, measured from the base of the plant to the highest point of the canopy, when 50% of plants had ripe fruits); 2) number of days to flowering (DFL, calculated from the sowing up to the time when at least half of the plants had at least one open flower). Regarding the fruit characteristics, the following items were evaluated: 1) total number of fruits (TNF); 2) length of the fruit (LF); 3) diameter of the fruit (DF); 4) fruit weight (FRW); 5) days to fruiting (DFR) and 6) days to the change of maturity stage of the fruits (DMF). For the analysis of LF, FRW and DF, the means from five randomly selected ripe fruits were used. LF and DF were measured with a digital calipers, expressed in millimeters (mm). FRW was equal to the mass of the fruits obtained in grams (g) carried out on an analytical balance. DFR was calculated from the transplanting up to the time when 50% of plants had ripe fruits in the first and second bifurcations, and DMF was obtained by marking the fruits, just after setting, with colored woolen yarns and by counting the number of days for color change from its initial stage to the next, until complete ripeness.

Qualitative data weren’t analyzed. The quantitative data’s were analyzed multivariate analysis with SPSS. Means were grouped according to Duncan test.

Results and discussion

Quantitative traits

There was a significant difference for all of the studied traits, assessed by Duncan test ($p>0.05$) (Table 1). Means for plant height separated the genotypes, in which the highest means were 77.3 cm (MKU-86) and the lowest one 10.2 cm (MKU-84). Neitzke et al. (2010), reported that this feature is of great importance when it comes to ornamental plants, since smaller plants are preferred and attractive for cultivation in pots.

In relation to the length of the fruit, the highest means ranged from 77.6 mm (MKU-69) to 82.7 mm (MKU-86) and the lower ones varied from 14.0 mm (MKU-97) to 17.6 mm (MKU-43); for the diameter of the fruits, the highest means ranged from 39.0 mm (MKU-72) to 44.5 mm (MKU-19) and the lowest ones varied from 6.0 mm (MKU-52 and MKU-104) to 6.7 mm (MKU-97); for fruit weight, the highest means were between 14.9 g (MKU-69) and 16.6 g (MKU-75) and the lowest ones ranged from 0.6 g (MKU-97) to 1.3 g (MKU-106). Fruits of

smaller size and lower weight are ideal for ornamental purposes, due to the small size of the plants.

Table 1. Data and means for eight quantitative characters of the 32 genotypes of Capsicum spp. from the germplasm collection of the Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture.

Genotypes	Species	HGT (cm)	TNF	LF (mm)	DF (mm)	FRW (g)	DFL	DFR	DMF
MKU-18	*C. annuum*	35.3 i	23 def	37.8 ef	29.5 k	11.8 m	33 b	52 efg	67 bc
MKU-19	*C. baccatum*	52.3 kl	46 jk	43.7 gh	44.5 n	13.3 n	74 l	81 l	96 l
MKU-30	*C. chinense*	28.0 gh	7 a	48.7 ij	27.7 k	8.3 k	90 o	110 o	139 p
MKU-35	*C. annuum*	19.5 cdef	57 l	27.1 c	16.1 fgh	1.9 bcd	54 g	64 i	74 def
MKU-43	*C. annuum*	24.5 efg	36 ghi	17.4 a	14.4 def	1.6 bc	83 n	102 n	120 o
MKU-44	*C. annuum*	21.7 cdef	97 m	25.3 bc	10.2 b	1.1 ab	68 jk	87 m	102 m
MKU-45	*C. annuum*	44.1 j	44 ijk	52.7 kl	18.7 i	3.3 fg	41 e	48 cde	75 def
MKU-50	*C. annuum*	36.1 i	23def	34.7 de	33.6 l	5.8 I	33 b	71 j	92 jkl
MKU-52	*C. annuum*	56.5 l	117 n	26.0 c	6.0 a	1.3 ab	63 hi	68 j	88 ij
MKU-67	*C. annuum*	20.0 cdef	11 ab	28.0 c	35.1 l	13.7 n	54 g	72 j	95 kl
MKU-68	*C. annuum*	21.9 def	15 a-e	80.0 op	18.2 i	9.7 l	79 m	89 m	113 n
MKU-69	*C. annuum*	31.8 hi	19 bcde	77.6 o	21.6 j	14.9 o	70 k	76 k	93 jkl
MKU-71	*C. annuum*	31.7 hi	17 bcde	52.7 kl	22.3 j	8.3 k	49 f	61 hi	86 hi
MKU-72	*C. chinense*	34.3 i	13 abcd	40.3 fg	39.0 m	7.2 j	91 o	125 s	145 rs
MKU-73	*C. annuum*	25.0 fg	92 m	49.9 jk	10.5 b	1.9 bcd	42 e	50 def	71 cd
MKU-74	*C. annuum*	46.9 jk	23 def	45.7 hi	17.3 hi	5.1 hi	53 g	61hi	88 ij
MKU-75	*C. pubescens*	37.3 i	15 a-e	37.0 def	33.7 l	16.6 p	105 r	112 op	147 s
MKU-76	*C. annuum*	35.5 i	40 hij	28.7 c	12.7 cd	2.7 def	43 e	53 fg	77 ef
MKU-79	*C. annuum*	18.3 bcde	51 kl	33.3 d	11.3 bc	2.7 def	35 bc	45 c	72 de
MKU-82	*C. annuum*	16.0 abcd	34 ghi	25.0 bc	17.0 ghi	3.0 efg	34 bc	40 b	65 b
MKU-83	*C. annuum*	22.3 efg	30 g	42.7 gh	14.0 de	3.3 fg	37 cd	47 cd	75 def
MKU-84	*C. annuum*	10.2 a	33 gh	55.0 l	10.7 b	1.6 bc	28 a	33 a	58 a
MKU-85	*C. annuum*	19.4 cdef	37 ghij	42.7 gh	10.3 b	1.5 abc	40 de	53 fg	79 fg
MKU-86	*C. annuum*	77.3 m	17 bcde	82.7 p	15.3efgh	7.9 jk	42 e	55 g	82 gh
MKU-87	*C. annuum*	15.7 abc	50 kl	34.2 de	15.1 efg	3.7 g	43 e	62 hi	85 hi
MKU-92	*C. baccatum*	35.7 i	12 abc	65.3 m	15.3 efgh	4.8 h	95 p	118 r	142 pr
MKU-96	*C. annuum*	47.0 jk	42 hijk	70.3 n	11.0 bc	2.7 def	61 h	69 j	90 ijk
MKU-97	*C. annuum*	13.0 ab	16 a-e	14.0 a	6.7 a	0.6 a	32 b	40 b	63 b
MKU-101	*C. annuum*	13.3 ab	33 gh	43.0 gh	14.3 def	2.3 cde	65 ij	69 j	89 ij
MKU-104	*C. frutescens*	52.3 l	38 ghij	22.0 b	6.0 a	1.6 bc	113 s	126 s	152 t
MKU-106	*C. annuum*	24.0 efg	18 bcde	29.0 c	9.7 b	1.3 ab	83 n	90 m	112 n
MKU-115	*C. annuum*	23.0 efg	22 cdef	78.7 o	15.2 efg	4.9 h	53 g	59 h	86 hi
	Avarages	**29.9**	**35.3**	**43.5**	**18.2**	**5.2**	**58.9**	**71.5**	**94.3**

HGT =plant height; TNF=total number of fruits; LF= length of the fruit; DF= diameter of the fruit; FRW= fruit weight; DFL= days to flowering; DFR= days to fruiting; DMF= days to the maturity of fruit

Regarding precocity, the earliest accessions flowered between 28 days (MKU-84) and 32 days (MKU-97) and produced fruits between 33 (MKU-84) and 40 days (MKU-97 and MKU-82).

Qualitative traits

Twenty three genotypes (MKU-18, MKU-19, MKU-30, MKU-35, MKU-43, MKU-44, MKU-45, MKU-67, MKU-68, MKU-69, MKU-71, MKU-72, MKU-73, MKU-74, MKU-76, MKU-79, MKU-82, MKU-84, MKU-85, MKU-87, MKU-97, MKU-101 and MKU-106) showed intermediate growth habit, while 5 genotypes showed erect growth habit (MKU-50, MKU-52, MKU86, MKU-96 and MKU-104) and four genotypes (MKU-75, MKU-83, MKU-

92 and MKU-115) showed prostrate growth habit. According to Stommel and Bosland (2006), the choice of ornamental plants is directly related to the height of the plant, since consumers prefer the lower-sized plants for cultivation in pots, and the growth habit is associated with the size of the plants, therefore, they are more accepted in the market of ornamental plants.

Table 2. Some qualitative characters of the 32 genotypes of Capsicum spp.(Used IPGRI pepper descriptor)

Genotypes	GH	SC	LC	LS	PFL	PFR	SFR	PGY
MKU-18	5	1	2	1	5	3	3	+
MKU-19	5	1	3	1	3	1	4	+
MKU-30	5	1	3	1	3	1	4	+
MKU-35	5	1	4	3	7	2	1	+
MKU-43	5	3	6	2	7	2	2	+
MKU-44	5	3	5	3	7	2	1	+
MKU-45	5	1	3	1	5	1	1	+
MKU-50	7	1	4	2	5	2	2	+
MKU-52	7	1	4	3	7	2	1	+
MKU-67	5	1	4	2	5	1	5	-
MKU-68	5	1	4	2	3	1	1	+
MKU-69	5	1	4	2	7	2	3	-
MKU-71	5	1	3	2	7	2	3	-
MKU-72	5	1	3	1	3	1	4	+
MKU-73	5	2	4	3	7	2	1	+
MKU-74	5	1	3	2	7	2	3	+
MKU-75	3	1	4	3	3	1	2	+
MKU-76	5	1	3	3	7	2	3	+
MKU-79	5	2	3	3	7	2	3	+
MKU-82	5	1	3	3	7	2	3	+
MKU-83	3	2	3	3	3	1	3	+
MKU-84	5	1	3	3	7	2	1	+
MKU-85	5	1	3	3	7	2	1	+
MKU-86	7	2	4	2	5	2	1	+
MKU-87	5	1	4	3	7	2	3	+
MKU-92	3	1	4	3	3	1	1	+
MKU-96	7	3	5	2	7	2	1	+
MKU-97	5	1	4	3	7	2	1	+
MKU-101	5	2	4	3	7	2	3	+
MKU-104	7	1	3	1	7	2	1	+
MKU-106	5	1	7	3	7	2	3	+
MKU-115	3	2	3	3	3	1	1	+

GH= growth habit; SC= stem color; LC= leaf color; LS= leaf shape; PFL= flower position; PFR= fruit position; SFR= fruit shape; PGY= pungency

The color of the corollas were showed different color some of them showed figure 1.

Figure 1. Some of genotypes flower shape and corolla color (Left to right, upper row MKU-19, MKU-30, MKU-35, MKU-43 MKU-44, bottom row MKU-67, MKU-68, MKU-69, MKU-71 MKU-74)

As for flower position, four genotypes (MKU-18, MKU-45, MKU-50, MKU-67 and MKU-86) showed an intermediate flower position, 8 genotypes (MKU-19, MKU-30, MKU-68, MKU-72, MKU-75, MKU-83, MKU-92 and MKU-115) showed pendant flower and the other 19 accessions were erect.

Genotypes MKU-19, MKU-30, MKU-45, MKU- 67, MKU-68, MKU-72, MKU-75, MKU-83, MKU-92 and MKU-115 produced pendant fruits, genotypes MKU-18 produced fruits in intermediate position and the other genotypes produced erect fruits. According to Mavi (2013), erect fruits are more used for indoor decoration and the fruit shape on the plant is related to the extension of the ornamental effect.

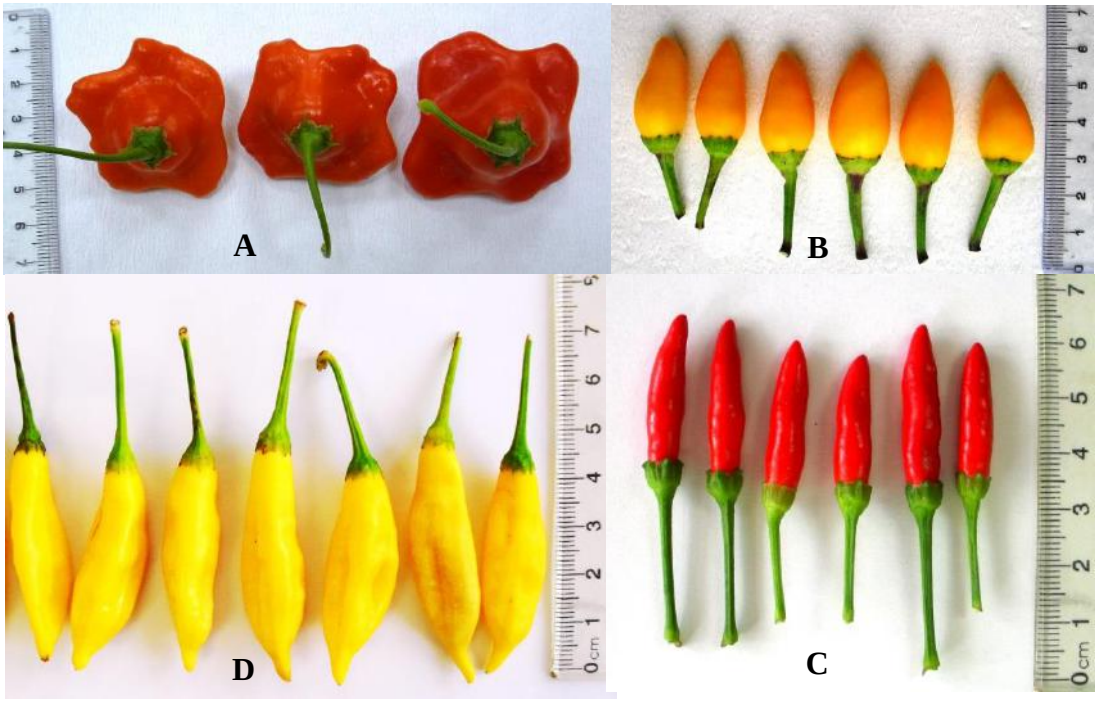

Figure 2. Some genotypes fruit shapes and, color A: MKU-19, B: MKU-35, C: MKU-52 and D: MKU-92

Considering the fruit shape, two genotypes were nearly round, eleven genotypes were triangular, three genotypes were campanulate, one genotype was cylindrical, and the other genotypes were elongated (Table 2 and Fig. 2).

The analysis of pungency of the fruits identified that only three (MKU-67, MKU-69 and MKU-71) of 32 genotypes are not pungent. The absence of pungency is important due to the use of these plants in environments that can be reached by children and genetic material to breeding.

Conclusion

A large number of tested pepper varieties differ in morphological and sensor characteristics (including colour), which determines the possibility and the mode of their usage. Not all pepper genotypes can be recommended for ornamental production, but must first be verified in production, through a series of agronomic and industrial pod plant demands. The greatest value of the genotypes tested, is their high content of genetic diversity, therefore all of the genotypes can be use for breeding program the potted ornamental plants.

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EFFECTS OF PREHARVEST SOME APPLICATIONS ON CRACKING AND FRUIT QUALITY OF '0900 ZİRAAT' SWEET CHERRY CULTIVAR

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Abstract

Turkey is an important producer and exporter of sweet cherry in the world. The most important sweet cherry variety in Turkey is 0900 Ziraat. The rain that falls during ripening period causes cracks in cherry fruits and impairs the quality of the fruits. Many applications are made by cherry producers to reduce the losses caused cracking. In this study, 0900 Ziraat cultivar grafted onto mahaleb (*Prunus mahaleb* L.) rootstock was used, pre-harvest calcium chloride ($CaCl_2$) and sucrose applications were made to determine the effect of fruit quality parameters, and cracking was examined. This study was carried out between the years of 2011 and 2012 in Eğirdir (Isparta-Turkey). Sprays of $CaCl_2$, sucrose and $CaCl_2$+sucrose were applied 7, 14 and 21 days before harvest. Also water was sprayed to the trees as the control application. Examples of fruit harvested were examined; some fruit quality criteria and artificial cracking tests were made. Treatment results affected some fruit quality criteria in the study. In terms of cracking index of the study, all three applications during the first year of experiment were not found to have an important effect on cracking. But all the trials compared to the control in the second year of study were determined to be effective for reducing cracking.

Keywords: *Prunus mahaleb* L.,calcium chloride, sucrose, cracking index

Introduction

Turkey is one of the leading countries in the world in terms of sweet cherry production and export. The positive developments that have taken place in export in recent years have positive impact on both production and quality. According to 2012 data, Turkey produced 480.748 tons and exported 46.477 tons (Faostat, 2014). Although there are different early and late ripening varieties in Turkey, mostly 0900 Ziraat is cultivated. 0900 Ziraat is the most important variety not only in production but also in export (Öztürk et. al., 2005).

In most of the regions in the world where sweet cherry is produced, cracks resulting from pre-harvest rainfall pose a serious problem (Sekse, 1995), limiting the number of sweet cherry production areas. Besides, cracking in sweet cherry eliminates fruit marketability. In subsequent stages, it incubates different diseases in cracked tissues, adversely affecting healthy fruits around the unhealthy ones. Intensive studies have been carried out with the aim of developing durable fruits, increasing resistance by means of different preparations, and providing mechanical protection so as to prevent fruit cracking in sweet cherry.

It is reported that there are a great number of reasons for cracking in sweet cherry. The most common idea is the crack of fruit skin as a result of surface tension caused by rainwater absorption through fruit skin (Verner and Blodgett, 1931; Sawada, 1931). In spite of differences between cherry varieties, fruits are susceptible to cracking especially 10 to 25 days before the date of harvest. The said susceptibility increases during the optimum harvest time (Christiensen, 1973). It is seen that, in some extreme years when cracking damage is high, more than 80% and even all of the fruits are lost (Christiensen, 1996; Csiszár, 2004).

Located in the cell wall structure, calcium is one of the main components functioning in the creation of plants main skeleton. It is possible to say that, calcium is particularly used in the studies aimed at reducing fruit cracking (Shear, 1975; Huang et al., 2005). Calcium leads to a change in fruit cuticular membrane and an increase in epidermal layer density (Marschner, 1995). Accordingly, calcium reduces cell membrane permeability and thus water absorption in sweet cherry (Verner, 1940). In other words, as the amount of calcium in fruit increases, transports through cell membrane decrease. On the other hand, resistance to cracking increases as the cell wall gains strength (Yamamoto, 1973).

Although some studies demonstrate that pre-harvest calcium applications are not successful in reducing fruit cracking in sweet cherry (Bangerth, 1968; Looney, 1985), there are many researches proving the opposite (Callan, 1986; Meheriuk et al., 1990; Yamamoto et al., 1992; Brown et al., 1995; Wermund et al., 2005). It is known that calcium applications affect not only cracking in fruit but also fruit quality criteria. $CaCl_2$ is one of the most widely-used substances for preventing cracking in fruits. Some researchers report that, in case of sweet cherry, applications containing salt or sucrose help balancing internal and external osmotic tension and also slow down the movement of rainwater absorbed through fruit (Schared and Sun, 2006.) Wójcik et al. (2013) applied $CaCl_2$ and $CaCl_2$+sucrose to Early Burlat variety in 2 different locations before harvest and found out that the applications reduced cracking. It is reported that $CaCl_2$ applied to 0900 Ziraat variety 10, 20 and 30 days before harvest decreased cracking by 62% compared to the control application (Erogul, 2014). Again, Demirsoy and Bilgener (2000) state that, as a widely-grown variety in Turkey, 0900 Ziraat is more resistant to cracking than sweet cherry varieties of Lambert and Van and has a thicker cuticle and epidermal layers.

This study is aimed at determining the effects of pre-harvest $CaCl_2$ and sucrose treatments on 0900 Ziraat sweet cherry variety cultivated under the conditions of Eğirdir, Isparta, Turkey.

Materials and methods

This study was conducted in Fruit Research Institute located in Eğirdir district of Isparta province between the years of 2011 and 2012. In the study, productive sweet cherry trees grafted onto Mahaleb (*Prunusmahaleb* L.) rootstock were used. Soil analysis results of the experimental orchards are given in Table 1.

As a high-quality sweet cherry variety having late ripening time, 0900 Ziraat has a very large, heart-shaped fruit. It has very firm texture and bright dark fruit skin (Akgül et al., 2005). Differences were observed between pre-harvest regional rainfall amounts of the two years of trial. Isparta's long years rainfall average for June is 31.7 mm. In 2011, monthly rainfall average for June was 46.5 mm, meaning an increase of 46.6% compared to long years average (Anonymous, 2015a). However, in 2012, monthly rainfall average for June was 22.3 mm, 31.6% lower than long years average. (Anonymous, 2015b).

Table 1.Soil analysis results of the cherry orchards

	Results	
Parameter	**0 - 30 cm**	**30 – 60 cm**
Saturation (%)	55	44
Salinity (ds/m)	0.307	0.242
pH	7.93	7.95
Lime (%)	14.67	14.67
Organic matter (%) (Smith Weldon)	2.23	1.12
Phosphor (Olsen-ICP) (ppm)	47	19
Potassium (A.Acetate– ICP) (ppm)	254	130
Calcium (A.Acetate – ICP) (ppm)	3665	3725

Magnesium (A.Acetate – ICP) (ppm)	421	204
Iron (DTPA – ICP) (ppm)	14.7	19.2
Copper (DTPA – ICP) (ppm)	5.8	2.3
Manganese (DTPA – ICP) (ppm)	4.6	6.6
Zinc (DTPA – ICP) (ppm)	1.6	0.5

$CaCl_2$, sucrose, $CaCl_2$+sucrose and water (control) were applied 7, 14 and 21 days before harvest depending on the weather (Table 2). $CaCl_2$, sucrose and $CaCl_2$+sucrose were sprayed to trees so that 0.5% solution of each material was applied per hectare (Wójcik et al., 2013).

Table 2.Treatment and harvest dates of the experiment.

	Date	
	1st year	**2nd year**
1stTreatment	29.06.2011	06.06.2012
2ndTreatment	05.06.2011	13.06.2012
3rdTreatment	12.06.2011	19.06.2012
Harvest	19.06.2011	27.06.2012

Fruits were harvested on 19.06.2011 and 27.06.2012 in the 1st and 2nd year of trial, respectively. 20 sweet cherry fruits to represent each tree were sampled for pomological assessments. On the other hand, for cracking studies, 50 sweet cherry fruits were sampled to represent each tree. For the purpose of determining cracking index, fruits were immersed in distilled water having a temperature of 20 ±1 °C in 2-liter vessels. Checking every 2 hours, cracked fruits were counted and put aside, while the remaining ones were re-immersed. At the end of 6 hours, cracking index was determined (Christensen, 1972).
In sampled fruits, fruit weight (g), fruit flesh firmness (N), pH, total soluble solids (TSS), and titratable acidity (TA) (g/100 ml) were set. Fruit flesh firmness was measured by penetrating with a tip having a diameter of 6 mm up to a depth of 8 mm at a speed of 10 cm/min. In the measurement, a texture analyzer (Güss FTA Type GS14 Fruit-Texture Analyser Model, Strand, South Africa) was used. TSS and TA were measured by digital refractometer and digital buret and digital pH meter (Hanna pH 330 model, WTW, Germany), respectively, after extracting juice from sample fruits. Ripening index was obtained by dividing TSS value by TA value. For measuring fruit skin color, a Minolta CR-400 (Konika Minolta Inc., Japan) chroma meter was used only in 2012. In the research, Randomized Block Design was replicated 5 times. Data obtained from the trial were put to an analysis of variance, using the JMP statistical software. Differences between varieties were grouped according to the LSD multiple comparison test ($P \leq 0.05$; $P \leq 0.01$; $P \leq 0.001$).

Results and discussion

There are numerous criteria determining sweet cherry fruit quality. These criteria are important for better marketability of products. Effects of the applications in this trial on fruit quality criteria are given in Table 3. In terms of the effect of applications on fruit weight, there was no difference in 2011. However, in 2012, sprays of $CaCl_2$, sucrose and control led to an increased fruit weight. Fruit flesh firmness is a crucial criterion for the marketability of sweet cherry. It was found that applications affected fruit flesh firmness in both years. Fruit flesh firmness increased in the 1st year of trial with sprays of $CaCl_2$, $CaCl_2$+sucrose and control, and in the 2nd year with sprays of $CaCl_2$ and $CaCl_2$+sucrose containing calcium. These findings comply with those obtained by researchers such as Rupert et al. (1997), Demirsoy and Bilgener (1998), and Erogul, (2014). Fruit flesh firmness is seen to be lower in

both years only in sprays of sucrose. For pH, control application had a higher value than the other three applications in the 1st year. However, in the 2nd year, no difference was detected. TSS differences were observed in the 1st year but not in the 2nd year. In the 1st year of application, sprays of $CaCl_2$+sucrose led to an increase in the TSS value. Besides, sprays of $CaCl_2$ and sucrose produced a higher TSS value than those of control. These findings are in accord with the findings that sprays of Ca increase the TSS value (Rupert et al., 1997; Demirsoy and Bilgener, 1998; Vangdal et al., 2008). Applications affected TA in both years. The applications which were significant in terms of TA values were sprays of sucrose and $CaCl_2$+sucrose in the 1st year and sprays of sucrose in the 2nd year. As can be seen, sprays of sucrose increased fruit TA in both of the years. Given the ripening index, a higher value was obtained from sprays of $CaCl_2$+sucrose and control in the 1st year and from sprays of $CaCl_2$ and control in the 2nd year. In both years of trial, control application's ripening index was higher. Ripening index was higher in ripe fruits compared to unripe fruits (Singh and Khan, 2010).

Table 3.Effects of $CaCl_2$, sucrose, $CaCl_2$+sucrose and control treatments on fruit weight (g), fruit flesh firmness (N), pH, TSS(%), TA (g/100 ml) and ripening index values. * : $P \leq 0.05$, ** : $P \leq 0.01$, *** : $P \leq 0.001$, NS : non Significance

	Fruit Weight (g)		Fruit Flesh Firmness(N)		pH		Total Soluble Solids (%)		Titratable Acidity (g/100 ml)		Ripening index	
Treatment	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
$CaCl_2$	7.69	8.98 a	12.25 a	10.4 ab	3.98 b	3.52	17.93 b	15.60	0.79 b	0.73 b	22.56 b	21.50 a
Sucrose	7.76	9.13 a	9.59 b	9.9 b	3.94 b	3.49	17.97 b	15.30	0.85 a	0.82 a	21.23 c	18.90 b
$CaCl_{2+}$ Sucrose	7.63	8.15 b	12.40 a	10.7 a	3.97 b	3.41	19.63 a	15.43	0.84 a	0.74 b	23.25 a	20.34 b
Control	7.30	8.77 ab	12.55 a	10.1 b	4.06 a	3.51	17.10 c	15.07	0.75 c	0.72 b	22.92 ab	21.21 a
Significant	NS	*	***	**	*	NS	***	NS	***	*	***	*
LSD		0,70	0,33	0,48	0,04		0,45		0,02	0,06	0,39	1,91

Fruits skin color and appearance are among the most important characteristics affecting customer choice. As is seen, L* value, as a determinant of lightness, is higher in sprays of $CaCl_2$ and $CaCl_2$+sucrose (Table 4.). On the other hand, a* value as the level of fruit skin redness is higher in 3 applications other than sprays of sucrose. In both years, applications containing $CaCl_2$ produced fruits having a redder and brighter skin. Fruit skin was lighter in sprays of $CaCl_2$ and $CaCl_2$+sucrose, where hue angle (h°) value was higher, but darker in sprays of sucrose and control. If color values are considered as a whole, it is possible to say that applications containing $CaCl_2$ delayed harvest.

Table 4. Effects of $CaCl_2$, sucrose, $CaCl_2$+sucrose and control treatments on fruit skin color (L*, a*, b*, C* and h°) values. * : $P \leq 0.05$, *** : $P \leq 0.001$

	Fruit Skin Color				
Treatments	**L***	**a***	**b***	**C***	**h°**
$CaCl_2$	30.45 a	25.11 a	10.20 a	27.12 a	21.72 a
Sucrose	29.17 bc	20.19 b	7.33 b	21.48 b	19.83 b
$CaCl_{2+}$ Sucrose	30.18 ab	25.41 a	10.16 a	27.38 a	21.49 a
Control	29.14 c	21.11 a	7.90 b	22.55 b	20.41 b
Significant	*	***	***	***	***
LSD	1,02	1,83	2,40	2,40	0,93

In spite of cracking that occurs in certain years, 0900 Ziraat is resistant to cracking (Akgül et al., 2005). Differences between applications during the 1st year of trial were not found to be statistically significant. On the other hand, in the 2nd year, sprays of $CaCl_2$, sucrose and

$CaCl_2$+sucrose had lower cracking index values compared to the control (Table 5). Of all applications, the lowest cracking index was obtained from sprays of $CaCl_2$ (10.60). These findings are in accordance with those obtained by Wójcik et al., (2013) in Early Burlat sweet cherry variety. It was reported that resistance to cracking increased as fruit flesh firmness increased and thus fruits with higher flesh firmness were more resistant to cracking than those with lower flesh firmness (Christensen, 1996). According to the data obtained, in sprays of $CaCl_2$ and $CaCl_2$+sucrose, fruit flesh firmness was higher, while cracking indices were lower, showing the relationship between fruit flesh firmness and cracking. Given the entire trial, cracking index values of the 1st year were higher than those of the 2nd year. The reason is thought to be the pre-harvest rainfall in 2011 that occurred 46.6% (Anonymous, 2015a) higher than long years average.

Table 5.Effects of $CaCl_2$, Sucrose, $CaCl_2$+sucrose and control treatments on fruit cracking.* : $P \leq 0.05$, NS : Non Significance

Treatment	Cracking Index 2011	Cracking Index 2012	Decrease % in comparison with control (%) 2011	Decrease % in comparison with control (%) 2012
$CaCl_2$	16.00	10.60 b	96.39	48.91
Sucrose	23.29	14.20 ab	140.29	64.55
$CaCl_{2+}$ Sucrose	17.24	11.20 ab	103.88	50.91
Control	16.62	22.00 a	100.00	100.00
Significant	NS	*		
LSD		5,12		

Conclusion

In this study, pre-harvest sprays of $CaCl_2$, sucrose, $CaCl_2$+sucrose and water were applied to 0900 Ziraat sweet cherry variety to analyze the effects of sprays on fruit cracking and quality criteria. As a result of the study, sprays of $CaCl_2$ positively affected fruit weight and fruit flesh firmness. Sprays of $CaCl_2$ enabled to produce firmer and more appealing fruits. In terms of cracking index data, no difference was observed between the data obtained in the 1st year. However, in the 2nd year, $CaCl_2$ and/or sucrose reduced cracking. In recent years, there has been an increase in the amount of precipitation in Turkey during the period of ripening and harvest for 0900 Ziraat sweet cherry variety. Even though being resistant to cracking as a variety distinguished in terms of production and export, preventive measures need to be taken against cracking, including pre-harvest sprays of Ca and some other minerals. In order to get the most appropriate result, similar studies may be repeated with different preparations, different doses, different application times, and combined applications.

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DETERMINATION OF THE OPTIMUM FERTILIZER QUANTITY BY THE SOIL ANALYSIS IN TURKEY

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Abstract

Soil analysis is used to determine the needs of the soil fertilizer. A good fertilization, determination of the amount and the type of fertilizer needed by the plant at the right time according to the technique of this fertilizer is to be buried.

One of the inputs that affect most of the increase in efficiency at agricultural production is manure. In order to increase the incomes of farmers and crop production due to increased efficiency in mind, to use the correct type and amount of fertilizer is inevitable.

A significant portion of the material to be used in research, in Konya province made from wheat breeding survey of the farms the data to be obtained. By going to the company by way of interviews with operators in the sample data of 2013 and 2014 production period is finished the questionnaire.

Enterprises grow wheat for research; as soil analysis and make those checks is determined in two sets. The survey will be administered enterprises are determined by simple random sampling method (SRS). In the process of data analysis, SPSS or Eviews software package is used.

Keywords: Analysis of Soil, Wheat Breeding Agricultural Enterprises, Fertilizer.

Introductıon

The content of soil and plant nutrients yield ability to increase the agricultural production is important. Plants need the plant nutrients in the soil to grow. If there araen't enough nutrients in the soil for the cultivation of plants, the appropriate type and amount of fertilizer must be given to close the gap. If the soil is deprived of essential nutrients and fertilizer contribution yet, yield losses are seen after a while.

In this regard, soil analysis is used in determining the fertilizer required by the soil. A good fertilization, determining the amount and the type of fertilizer the plant needs at the right time according to the technique of this fertilizer is to be buried. The type and amount of fertilizer required for soil laboratories specializing in various soil samples taken from a certain land is determined. In favor of soil analysis, food ingredients required by the soil can be determined correctly.

One of the most affecting inputs increasing the yield in agricultural production is fertilizer. The share of fertilizers in productivity growth varies depending on various conditions. Fertilizer prices in the available resources to farmers in those with significant increases are required to use the most efficient and shipped to adopt new technologies. Otherwise, while fertilizer costs and the type and amount of fertilizer that farmers use unconsciously are increased, the efficiency to be obtained will decrease. In this context, soil analysis with minimal impact from rising costs and the efficiency of determining the appropriate type and amount of fertilizer will be provided in a significant increase in earnings. It is important in terms of proper fertilization techniques to conserve natural resources.

Turkey is a country with a growing population structure and that Turkey's annual growth rate of population for 2013-2014 is ‰13.3 (Turkstat, 2015). This is to increase the agricultural production for an adequate and balanced nutrition of the population, it is necessary to

strengthen the capacity for diversity and efficiency. Beside preserving natural resources sustaining their potential must be provided. Wheat has taken its place among the most important crops to feed our people in our country. There are factors that appears wheat production in Turkey and the countries outside Turkey such as drought, war, natural disasters and so on. When wheat prices with low elasticity of demand reduction for reasons both within and outside the country is going up. Wheat breeding have been selected for the most important area of Konya province in our country as the research areas. Approximately one third of agricultural land devoted to wheat production in Turkey.

The current status of wheat breeding in Konya is examined to provide solutions and suggestions to consciously use the important input putting out fertilizer efficiency and its impact on the income used as a result of making soil analysis in wheat breeding according to the analysis report. Also, an agricultural policy of the State applied to the soil analysis is to support the monitoring.

Materıals and methods

A significant portion of the material used in the survey, which was composed of wheat farming in the area of agriculture to data obtained by the enterprise survey. Survey by interviewing enterprises questionnaire forms have been filled out by going into enterprise.

Data for the period from 2013 and 2014 production of agricultural enterprises were collected by questionnaire. In addition to the primary data obtained well as research findings that were previously made on the subject, which has benefited from the secondary data records and published by various organizations.

Studies and surveys were identified and then a set of 110 enterprises were to be implemented by the random sampling method (SRS) (Yamane, 1967). Enterprises who have their soil analyzed grow wheat 82 enterprises and 28 enterprises taking out soil analyzes were determined in two sets.

The purpose of the study, scope and characteristics of agricultural holdings was arranged by considering the questionnaire. Questionnaires and interviews with enterprise owners were completed by researchers. With the data obtained by questionnaire population structure of the agricultural enterprises, education status, working widths, ownership, crop production and yields, soil analysis level of consciousness, to identify the relationship between the organizations concerned with agriculture, soil analysis made/not made my reasons, producers of fertilizer application pay attention to the situation and so on. As it is aimed to determine the social and economic indicators.

The information in the questionnaire data entry is made in the Excel environment. The primary data analysis using SPSS and Eviews entered into the computer program and evaluated in the process of statistical tables were prepared (Eviews, 2014).

Soil analysis for comparison during separate checks and make an appropriate econometric model that two groups work was conducted. A fertilizer variable is effecting the production considered the efficiency of the production modeling work was done.

In this study, Let us assume that hipotetical exponential model is as follows;

$$Y_i = \alpha X_1^{\beta_i} e^{u_i}$$

When we take a logarithm for e base both two side,

$$\ln Y_i = \ln \alpha + \beta_1 \ln X_1 + u_i \ln e$$

In this model, ln is natural logarithm and according to the e base that e=2.718.

At the above model, we arrange these variables and rewrite this model is as follows;

$\alpha = \ln \alpha$, $\ln Y_i = Y_i^*$ ve $\ln X_1 = X_1^*$

The new arranged model is as follows;

$$Y_i^* = \alpha + \beta_1 X_1^* + u_i$$

The new model is used as a linear model. After that, we can estimate the parameters by Ordinary Least Squares (OLS) (Gujarati, 1999).

Results and dıscussıon

Socio-economics indicators

Soil analysis based on the use of fertilizers and soil analysis of the company are formed in two groups to make use of fertilizers in wheat production, and comparisons are made.

Not taking of soil analysis 28 enterprises and the taking of soil analysis 82 enterprises built the business surveys are conducted. The socio-economic factors are analyzed and the results are given in Table 1.

71.4% of those taking soil analysis, observed that takes place in the 40-69 age range, while 89% of those who perform the soil analysis results were in the 30-69 age range.

Manufacturers have been examined in accordance with the distribution of education. The proportion of primary school graduates in each two groups are between 57% and 65%. 28.6% of those not taking soil analysis, performing soil analysis is 13.4% of the people graduates who are high school graduates.

Enterprises are examined by the distribution of the time they are engaged in farming. It was found that both groups have a duration of between 10 and 49 years. The proportion of enterprises engaged in the enterprise of taking over 50 years of farming ranged between 5% and 11% at taking and not taking soil analysis.

Land ownership of manufacturers have a fairly high rate of 86-88%. While manufacturers who do not take soil analysis have the average of 24 land size, producers who take the soil analysis have around an average farm size of 203. In both groups highlights the height of land ownership. It was observed that the leased land ranged 12-13% rate. While the average size of leased land analysis for not taking soil analysis is 24 da for taking soil analysis is 117 da.

While tractor ownership is 97% not taking soil analysis in the group who is 100% in the group not taking soil analysis. While the harvester ownership is 7.3% for taking soil analysis group there is no harvester the another group. Tractor ownership (1 tractor owner) was 93% for not taking soil analysis, 73% of those who make analysis. 2 and over tractors ownership of those for taking soil analysis were found to be higher. For taking soil analysis with enterprises has 7.3% harvester ownership.

Enterprises surveyed in those areas doesn't make soil analysis of wheat grown an average of 13.9, while those built in the analysis were found to be 139.7. Wheat yields in favor of those who make analysis of about 16 kg / da more.

Table 1. Socio-economic indicators for taking soil analysis and not taking soil analysis

	Not taking soil analysis		Taking soil analysis	
Socio-economic indicators	Number	%	Number	%
Gender				
Male	28	100,0	82	82,0
Female	0	0,0	0	0,0
Total	28	100,0	82	82,0
Age				
20-29	2	7,1	8	9,8
30-39	3	10,7	15	18,3
40-49	4	14,3	24	29,3
50-59	10	35,7	25	30,5
60-69	6	21,4	9	11,0
70-79	3	10,7	1	1,2
Total	28	100,0	82	100,0
Education				
Literate	0	0,0	0	0,0
Primary	16	57,1	53	64,6

Secondary	3	10,7	15	18,3
High School	8	28,6	11	13,4
College	0	0,0	0	0,0
University	1	3,6	3	3,7
Master-Doctorate	0	0,0	0	0,0
Total	28	100,0	82	100,0
The time dealing with farming (Year)				
Less than 9	2	7,1	3	3,7
10-19	6	21,4	20	24,4
20-29	4	14,3	11	13,4
30-39	3	10,7	22	26,8
40-49	10	35,7	22	26,8
50+	3	10,7	4	4,9
Total	28	100,0	82	100,0
Saving shape of the land				
Property field	26	86,7	82	88,2
Leased field	4	13,3	11	11,8
Total	30	100,0	93	100,0
Land size (da)				
Property field	24,4	50,1	203,2	63,6
Leased field	24,3	49,9	116,5	36,4
Total	48,7	100,0	319,7	100,0
Tractor (Number)				
1	26	92,9	60	73,2
2	2	7,1	18	22,0
3	0	0,0	1	1,2
Ownership haven't	0	0,0	3	3,0
Total	28	100,0	82	100,0
Harvester (Number)				
1	0	0,0	6	7,3
Ownership haven't	28	100,0	76	92,7
Total	28	100,0	82	100,0
Kind of field				
Watery land	21	75	64	78
Dry land	7	25	18	22
Total	28	100	82	100
Information for wheat				
Cultivated areas (da)	13,9		139,7	
Yield (kg/da)	403,6		419,39	
Sale price (Turkish Lira)	0,85		0,87	

Different Indicators

Manufacturer of a variety of ideas about the soil analysis are given in Table 2. How it was asked to determine the amount of fertilizer used in corn producer. Between 60% and 71% of farmers are seen to benefit from their experience, 39% stated that they did according to the fertilization of the soil analysis results. The highest share of wheat in determining the amount of fertilizer used in the enterprises surveyed were found to be their own experiences in both groups. The ratio of the company determines the amount of fertilizer according to soil analysis results is 39%.

Soil analysis done properly be said that the proportion of manufacturers to benefit from the fertilizer subsidy ranged from 82% to 71% in both groups.

While manufactırers who want to give support of manure as fertilizer in both groups ranged between 55% and 61% who want to give support as money in both groups ranged between 36% and 42%. Manufacturers would like manure support as a fertilizer. The proportion of enterprises that want the money the fertilizer subsidy is between 36-42%.

79% of producers who don't take a soil analysis, and do soil analysis, 50% producers who do a soil analysis of those checks to the analysis results obtained in the amount of fertilizer use products / yield believes that increase. 12.2% of manufacturers who stated that the change of soil analysis not increased efficiency. The proportion of enterprises believe will improve the efficiency of taking out soil analysis ranged from 50-79%. Those not taking out soil analysis, this rate is 79%.

Table 2. Different opinions belong to producers taking soil analysis and not taking soil analysis

Different opinions belong to producers	Not taking soil analysis		Taking soil analysis	
	Number	%	Number	%
Determining the amount of fertilizer used in Wheat				
To consult the agriculture directorate	2	7,1	1	1,2
To consult the place of purchase fertilizer	3	10,7	0	0
Getting around the idea	3	10,7	0	0
In my own experience	20	71,4	49	59,8
According to the results of soil analysis	0	0	32	39
Total	28	100	82	100
The suitability of the soil analysis done to take advantage of the fertilizer subsidy				
Yes	23	82,1	58	70,7
No	5	17,9	23	28
Other	0	0	1	1,2
Total	28	100	82	100
Giving how the fertilizer subsidy				
Fertilizer	17	60,7	45	54,9
Money	10	35,7	34	41,5
Other	1	3,6	3	3,7
Total	28	100	82	100
Soil analysis revealed that the amount of fertilizer to use the yield / efficiency will increase your faith				
Yes	22	78,6	41	50
No	6	21,4	31	37,8
Fixed	0	0	10	12,2
Total	28	100	82	100

Econometrics Model

Enterprises grow wheat as taking soil analysis and not taking soil analysis is determined in two sets at this research. In agricultural production among the most inputs affecting the yields increase is counted as a fertilizer.

During separate comparison checks for taking soil analysis and not taking soil analysis, multiple linear regression models were conducted.

The best double-logarithmic (log-log) model was represented by the results and the Least Squares (OLS) method was applied these data and given as follows.

The coefficient of determination (R^2), is known as one of the best indicators that describe the model (Johnston, 1991). R^2 is built on the model of the soil analysis was 27.8%. So, fertilizer as a independent variable indicate that explain the dependent variable of the yields rate of 27.8%.

In practice, we can expect the F calculated 3-4 times bigger than the F critical value. Otherwise, it is difficult to decide to comply with linearity. Accordingly, the FH = 30734> F0,05; 3; 78 = 2.68 hypothesis H0 is rejected and used for equation obtained at 95% confidence desired purposes.

Table 3. Statistics value for taking soil analysis				
Variable	Coefficient	Std. Error	t-Statistic	Prob.
Constant	3,692	0,414	8,909	0,000
Fertilizer	0,776	0,140	5,544	0,000
R-Squared	0,278			
F-statistic	30,734			
DW Stat	0,967			

R^2 is the model for not taking a soil analysis is 23.0%. So, fertilizer as a independent variable indicate that explain the dependent variable of the yields as a rate of 23.0%. Accordingly, the FH = 7.763> F0,05; 3; 24 = 3.01 The null hypothesis H0 is rejected and used for equation obtained at 95% confidence desired purposes.

Table 4. Statistics value for not taking soil analysis				
Variable	Coefficient	Std. Error	t-Statistic	Prob.
Constant	3,675	0,817	4,496	0,000
Fertilizer	0,758	0,272	2,786	0,009
R-Squared	0,230			
F-statistic	7,763			
DW Stat	1,102			

Conclusıon

Fertilizer and soil analysis in support of social enterprises in the study were examined in terms of economic and various opinions. Among the social situation of the firms surveyed are sex, age, education level, dealing with time farming, land tenure and size and so on. Also determine the amount of fertilizer used in wheat between various indicators, soil analysis commissioned the eligibility to benefit from the support of fertilizers, how it is given to the fertilizer support and soil analysis result to obtain fertilizer use product quantity / yield was examined submissions on that increase.

In the enterprise of taking over 50 years of farming who have their soil analyzed and haven't their soil analyzed ranged between 5% and 11%.

In both groups the height of land ownership is highlighted. While those taking the average size of leased land 24 da for not taking soil analysis, analysis of those 117 are built in.

Tractor ownership (1 tractor owner) was 93% for patients taking the soil analysis.

Enterprises surveyed in those areas weren't make soil analysis of wheat grown an average of 13.9, while those built in the analysis were found to be 139.7. Wheat yields, the analysis in favor of those who make about 16 kg / da more.

The highest share of wheat in determining the amount of fertilizer used in the enterprises surveyed were found to be their own experiences in both groups.

Manufacturers would like to the fertilizer as a support. The proportion of enterprises that want the money the fertilizer subsidy is between 36-42%. The support payment to producers of fertilizers in purchasing fertilizer will be more useful.

The proportion of enterprises believe will improve the efficiency of taking out soil analysis ranged from 50-79%. Monitıring of soil analysis support will be useful to decide next agricultural politics for Turkey.

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ASSESSMENT OF ANTIFUNGAL ACTIVITIES OF PLANT EXTRACTS FROM VITEX AGNUS-CASTUS L.

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Abstract

In this study, it was aimed to determine antifungal activity of methanol extract from *Vitex agnus-castus* L. which is growing naturally in Turkey. This plant was formerly classified in the family of *Verbenaceae*, but now phylogenetic classification situated the plant within the *Lamiaceae* family. *Vitex agnus-castus* was examined against 3 common plant pathogens which were *Alternaria solani, Rhizoctonia solani* and *Sclerotinia sclerotiorum* under *in vitro* conditions. Methanol extract of *Vitex agnus-castus* was dissolved with 50% acetone for each doses (50, 100 and 200 mg doses) and added inside the 100 ml PDA (40 ^{o}C). 5 mm diameter fungus mycelium discs were transferred to petri dishes used to keep PDA media (10 ml/petri dish). After that, petri dish were covered immediately and wrapped tightly with parafilm. They were incubated at 25±2ºC for 7 days in incubator. As a result of the study, the methanol extract of *Vitex agnus-castus* showed inhibitory effect against the mycelial growth of *A. solani*. The methanol extract inhibited mycelium development of *A. solani* depending on increasing doses, 27.73%, 32.98% and 40.08% respectively. In contrast, methanol extract of *Vitex agnus-castus* was not showed that inhibitory effect against mycelial growth of *R. solani* and *S. sclerotiorum.*

Keywords: *Vitex agnus-castus* L., Antifungal activity, *Alternaria. solani, Rhizoctonia solani, Sclerotinia sclerotiorum*

Introduction

Turkey is divided into 3 basic phytogeographic regions including Mediterranean, Iran-Turan, and European-Siberian region depending of its geographical location (Doğan and Mert, 1998). For this reason, it is prosperous in terms of plant diversity and endemism. Vitex agnus-castus L,which is a typical element of Mediterranean lemur vegetation, is a perennial plant in the family of Verbenaceae (Anonim, 2015). However, it has been involved in the family of Lamiaceae in the final classification.(Asdadi et al., 2014). *Vitex agnus-castus*, which is called with various names,such as,hayıt, acı ayıt, hayıd, and beş parmak otu , are the plants in the form of bush, rarely small trees, growing on the coast and rocky terrains (anonymous, 2015). Although the history of the information of usage of *Vitex agnus-castus* plant in medicine for healing wounds and piles extends back to at least years ago, (Chantaranothai, 2011) today, different parts of it are used to heal remenstrual, spasmodic dysmenorrhea, menopasual conditions and some discomforts, such as insufficient lactation ve acne (Maltaş et al., 2010). Besides, antioxidant, antimicrobial and antifungal activities of volatile oil and plant extracts of *Vitex agnus-castus* have been reported. Also it has been reported that volatile oil obtained from the seeds of *Vitex agnus-castus* exhibites a strong antifungal activity on *Candida* species (Asdadi et al., 2014), and *Vitex agnus-castus* methanol extracts including seven bacterial types have an antimicrobial effect against *Candida albicans* fungus (Maltaş et al., 2010). With this study, it has been revealed the antifungal activity of methanol extracts of *Vitex agnus-castus* plant growing normally in Turkey, on *Alternaria solani, Rhizoctonia solani and Sclerotinia sclerotium* plant pathogens leading to significiant losses in agricultural production.

Material and methods

Plant Materials

The Vitex agnus-castus plant materials used in this study have been picked up in August 2014, in Antalya province, and dried at room temperature in the shade. They have been stored until being used in trials.

Fungus Cultures

The fungi of plant pathogen used in the study (*Alternaria solani, Rhizoctonia solani* and *Sclerotinia sclerotorium*) were obtained from the stock cultures in Phytopathology laboratories of Department of Plant protection, Faculty of agriculture, Ahi Evran University.

Preparation of Extracts

For the purpose of obtaining of plant extracts, dried and ground plant materials of 100 g has been located in erlenmeyer of 1 lt, and methanol solvent has been added enough to cover it. It has been subjected to the process of rinsing through a orbital agitator at 120 rpm and room temperature for 24 hours.Then the extracts have been filtered through a filter paper. The solvents have been kept away by being evaporated at 400C by ratory evaporator. The remaining dry extracts have been dissolved with acetone, and finally, the different concentrations, such as 5% (50 mg), 10% (100 mg) and% 20 (200 mg), have been obtained for being used in our study (Kalkısım, 2012).

Antifungal effect of plant methanol extract

Obtained plant extract have been autoclaved in such a way that the last concentrations would be % 1 (10mg), %5 (50mg), %10 (100mg) and %20 (200mg),and then added to the sterile PDA's,which were cooled until 40^0. The PDA's have been transfered to 60 mm diameter petri vessels (in a way as being 10 mm.). The mycelium discs taken from 7-days fungus cultures previously have been transfered to 5 mm diameter petri vessels. After inoculation, fungus cultures have been subjected to incubulation at 25±2 0C for 7 days, and at the end of the period, developed radial mycelium have been taken (Hadizadeh et al., 2009). The development of mycelium has been calculated according to the formula given below (Pandey et al, 1982). As positive control, a standard fungicide has been used containing %50 captan. The trial was conducted in through four replications and repeated twice

$$MGI=100\times(dc-dt)/dc$$

MGI: percentage mycelial growth inhibition

dc; mycelial growth in control

dt; mycelial growth in treatment

Statistical analyses

The data were analyzed using the Analysis of variance (ANOVA) test. The means of treatments were grouped on the basis of least significant difference (LSD) at the 0.05 probability level. The SPSS 13.0 software was used to conduct all statistical analyses.

Results and Discussion

The effects of methanol extract of *Vitex agnus-castus* on the development of *Alternaria solani, Rhizoctonia solani* ve *Sclerotinia sclerotiorum* mycelium has been given below in Table 1 and Figure 1.

Although the effects differ depending on extract dose and diseases, it has been determined that methanol extract of *Vitex agnus-castus* has generally exhibited an activity on fungi. Methanol extract of *Vitex agnus-castus* has exhibited the highest effect on the development of *A. solani* mycelium with the highest rate of 40.08% (Figure 1).

Table 1. Effects on plant pathogenic fungi mycelium growth of *Vitex agnus-castus* methanol extracts

Application Dose	*A. solani*	*R. solani*	*S. sclerotiorum*
Negative Control	60,00 a*	60,00 a	60,00 a
Positive Control	0,00 e	0,00 b	0,00 b
50mg(%5)	43,66 b	60,00 a	60,00 a
100mg(%10)	40,21 c	60,00 a	60,00 a
200mg(%20)	35,95 d	60,00 a	60,00 a
LSD	**0**	**0**	**0**

* Means in the same column by the same letter are not significantly different to the test of ANOVA (a = 0.05).

The adverse effect on *A. solani* hasn't been realised on the development of mycelium of other important plant pathogen fungi that are *R. solani* and *S. sclerotiorum*. Even the highest dose of methanol extract of *Vitex agnus-castus* hasn't any effect on the development of mycelium of *R. solani* and *S. sclerotiorum.* However, through the trial observations, it has been determined that methanol extract of *Vitex agnus-castus* has caused development speed of mycelium of *R. solani* and *S. sclerotiorum* to be slowed down.

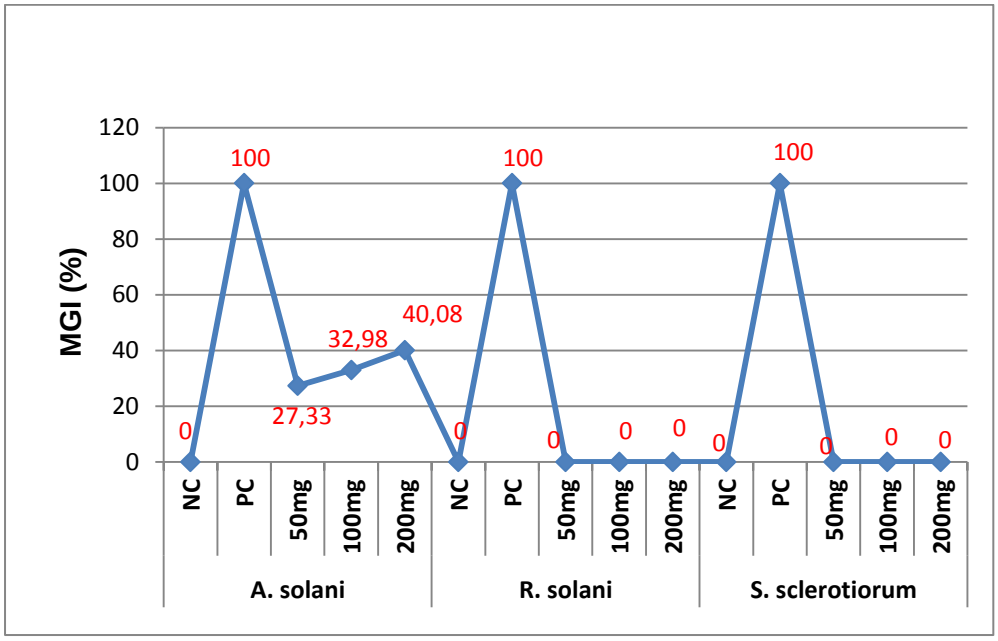

Figure 1. The inhibition ratio of methanol extract of *Vitex agnus-castus* on the development of mycelium of plant pathogen fungi.

The different effects of methanol extract of *Vitex agnus-castus* on pathogens is due to development differences of pathogens and tolerance degree of pathogens for the components contained in plant structure. The biological activity of vitex agnus-castus plant is due to the active compounds contained in its structure,such as, phenolic, flavonoid, diterpen and volatile oil (Stojković et al., 2011; Latovi et al., 2012). İt has been determined that 1,8-cineole compound isolated from volatile oil of vitex agnus-castus has been effective against aspergillus pathogen on fruit, and some volatile oil of it has exhibited important activity against some fungi and bacteria (Stojković et al., 2011). In another study,it has been reported that the methanolic extract of *Vitex agnus-castus* has exhibited important activity

against *Pythium ultimum* pathogen in the conditions of in vitro and in vivo (Švecová et al., 2013) and exhibited antioxidative and antimicrobial activity on tomato (Sarikurcu et al., 2009; Maltaş et al., 2010).

As a result, it has been revealed that methanol extract of *Vitex agnus-castus* hasn't been effective on development of mycelium of *Rhizoctonia solani* ve *Sclerotinia sclerotiorum* and exhibited antifungal activity against *Alternaria solani* pathogen. Today, importance of this and similiar studies has been increased since durability problem has been occured as a result of that synthetic fungicide used against plant pathogen fungi have damaged to the environment and people. Through this study, it has been revealed that vitex agnus-castus plant having different usage areas has exhibited antifungal activity on fungi.

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INVESTIGATION OF VIRULENCE AND PRESENCE OF MIKOVIRAL dsRNA ON LEUCOSTOMA SPP. ISOLATES OF THE CHERRY PRODUCTION AREAS IN THE AEGEAN REGION/TURKEY

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Abstract

Leucostoma (Cytospora) canker is one of the major diseases that is cause to desiccation in partially or in whole of cherry trees. It was determined the presence of Leucostoma canker on various stone fruit species in different regions of Turkey. In ABD Michigan state, two *Leucostoma personii* isolates were determined in peach orchards. There are no studies on the presence of dsRNA on Leucostoma species in Turkey. In this study, it was determined Leucostoma isolates of the containing dsRNA in cherry field of the Aegean Region/Turkey. For this purpose, *Leucostoma* spp. isolates which is derived from maximum cherry production in Aegean region was evaluated for virulence using branches test and apples test. According to these tests 26 isolates were selected for dsRNA analysis and experiment was performed in three repeats. dsRNA profile was identified in six isolates for the first repeat but for seconds only in three isolates were determined, whereas no dsRNA profile in third assay. This uncorfirmation results may be obtained because of desiccation and freezing-thawing. More detailed investigation should be carried out in the future.

Keywords: Leucostoma canker, *Leucostoma* spp., Cherry, dsRNA

Introduction

Cherry (*Prunus avium)* is a fruit tree from the *Roseceace* family that is naturally present in Giresun, the South Caucasus, the Caspian Sea and Northeast Anatolia. Cherry has a wide spread in the world. However, it is commercially produced in countries such as Turkey, Iran and Italy. Even though it is changing according to years and seasonal conditions, Turkey is the first producer for cherry production in the world and it has a right to say in cherry exports (Anonymous, 2014). There might be important losses in production of cherry due to some diseases and pests (Karaca, 1972; Gökçe et al., 1998, Spotts et al., 1990). The desiccation parts in the branches or on the whole tree is one of the reasons of these losses. (Karaca et al., 1972; Gökçe et al., 2011). One of the most important diseases that lead to desiccation tree or branches and the stools in cherry trees is the Leucostoma canker (Cytospora canker). Leucostoma (Cytospora) canker generally appears as depressed dark areas around the leave traces and eyes in annual stools and can kill all the stools with backward deaths (Biggs, 1989; Ogawa et al., 1995). Leucostoma canker was first detected by Stewart et al. on peach tree, which is one of the stone-nuclei trees in the western areas of New York in 1900 (Biggs, 1989). Following this, in 1909, Rolfs reported that the disease was observed in the USA-Missouri and Gussow reported another observation from Canada in 1912 as damages on peach trees (Biggs, 1989). Leucostoma canker was also known as Cytospora Cancer and Valsa Cancer (Hayova and Minter, 1998). The disease is widely observed in cherry, nectarine and peach trees in areas with colder winters in Canada and the USA. It was reported in many European countries, including Turkey, that the disease was widespread in peach, apricot and cherry trees (Hayova and Minter, 1998). Hammar et al. (1989) detected dsRNA in *L. persoonii* cytoplasm in peach trees in Michigan State, the USA, and the cultural characteristics were defined. According to these findings, it was observed that the isolates which contained dsRNA were in lighter colors, developed mycelial in less density, and there were melting on

the hyphae tips, and did not form pycnidia. Jensen and Adams (1995) isolated dsRNA's from the cytoplasm of *L. persoonii* isolate which was encoded as NC14.4A and obtained from the Northern Carolina, the USA. Adams et al. (1990) reported that they determined the VCG (Vegetative Coherence Group) variety which is important in the spread of hypovirulence in *L. persoonii* isolates collected from two different peach orchards in Michigan, the USA.
The studies conducted on Leucostoma are extremely limited edition, both Turkey and in other countries. In addition, although dsRNA is detected in *Leucostoma* spp. which is causing damage in peach trees (Hammar et al., 1989; Jensen et al., 1995, Adams et al., 1989), we could not encounter any studies on dsRNA existence in *Leucostoma* spp. which causes problems in cherry tree. For this reason, in this study, the mycelial development of the *Leucostoma* spp. isolates obtained from the cherries in the cities in Aegean Region was investigated in terms of branch test, apple test, and containing dsRNA. The apple test in *Leucostoma* spp. isolates and the dsRNA existence obtained from cherry trees were first investigated with this study.

Materials and methods

Materials

The main material of the study are 100 *Leucostoma* spp. isolates. One year old 'Napolyon' (0900 Agriculture) shoots and Apple (Granny Smith) were used in the virulence test. Upon virulence test, the selected isolates were perfomed with dsRNA analysis.

Methods

Virulence Tests

Inoculation to Cut Branch

The virulence test was performed in isolates with Scorza and Pusey (1984) method for Chestnut Blight (*Cryphonectria parasitica)*. This test was used in yearly 'Napolyon' cherry branches. The scar was formed in the tip of each branch and the isolates were located on the scar. The branches were kept in the climate chamber in flowerpots by using Cut Branch method.

Apple Test

The isolates located on the Granny Smith apple fruits were used in the virulence test (Hammar et al., 1989). Apple fruits were selected, washed and the surface disinfection was performed by using 70% alcohol. The disk was removed from the surface of the fruit by cork borer and isolates were put in these holes.

dsRNA Analysis

Branch and apple test results were subjected to SPSS analysis and the 26 isolates which showed high and low virulence were examined whether or not they had dsRNA. dsRNA isolation was performed according to the method developed and used for *C. parasitica* (Morris and Dodds, 1979; Robins and Griffin, 1997; Valverde, 1990). Isolates were isolated with phenol and chloroform and CF II column chromatography was conducted for dsRNA.

Results and discussion

Virulence Tests

Inoculation to Cut Branch

The virulence degrees of the 100 isolates were determined with the virulence tests performed with the cut branch method on 'Napolyon' cherry. Lesions varying between approximately 1

and 14 cm emerged on the bark tissue of the branches which were inoculated. There were also branches which did not have lesions (Figure 1).

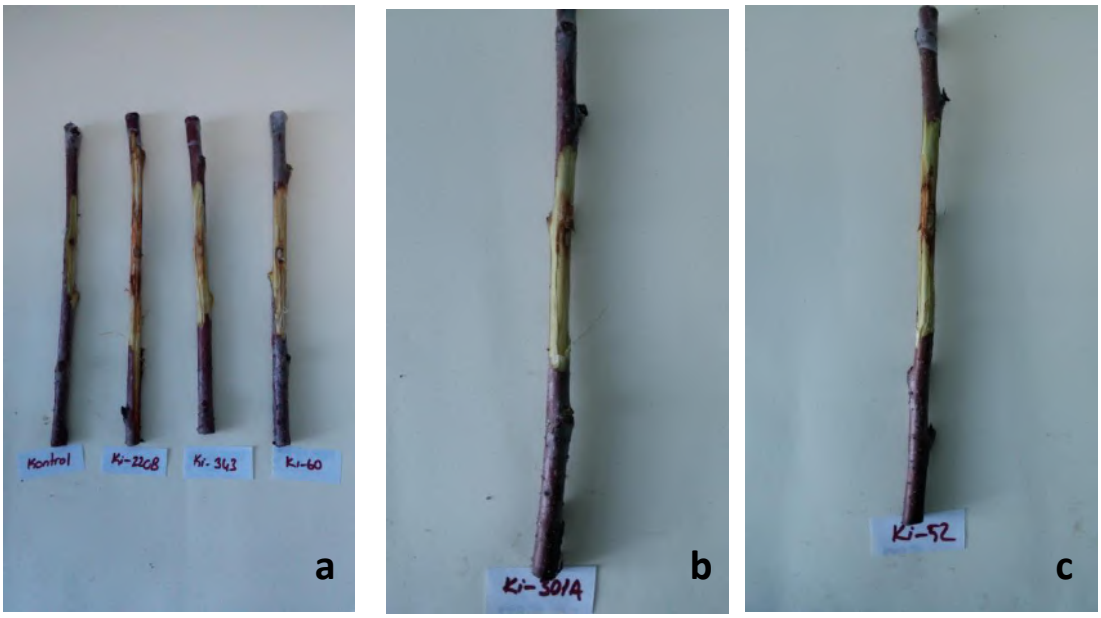

Figure 1. ‘Napolyon’ cherry created lesions by *Leucostoma* spp. a) High virulence reaction (Ki-222B) b) No indication of the lesion (Ki-301A) c) Low virulence reaction (Ki-52)

Upon the branch test, although 3 isolates (Ki-301A, Ki-194, Ki-343) did not show any lesions, all of the *Leucostoma* spp. isolates tested in the study conducted by Yılmaz (2013) were reported as being pathogen in cherry. In addition, it was reported in the same study that the plum, peach, apricot, almond species, which received cut branch test application, were sensitive to *Leucostoma* spp.

Apple Test

Inoculations were performed to evaluate the size of the lesions formed on apple fruit by 100 isolates obtained from *Leucostoma* spp. in terms of virulence, and it was observed that there were round, brown, soft and slightly depressed lesions in various sizes in the fruits (Figure 2).

Figure 2. Create different sized lesions on Granny Smith apple by *Leucostoma* spp.

The diameter of the lesions were measured five days after the inoculation and ten days after the inoculation. It was observed that the average lesion diameters on the fifth and tenth days were between 1,2-2 cm and 1,6- 3,8 cm, and iyt was also observed that there were no lesions on some fruits. Since the apple test with *Leucostoma* spp. has been applied for the first time in this study, the results were compared with those obtained in the study conducted with *C. Parasitica*.

In the apple test, while the diameters of some of the lesions formed on apple by *Leucostoma* spp. isolates were closer to each other in some groups, these values showed differences in some groups. While Bisiach et al. (1988) claimed that it was not reliable because the sizes of the lesions formed by virulent and hypovirulent isolates of the *C. parasitica* in apple test were closer to each other, Elliston (1985) and De Lange et al. (1998) found in the apple test results that the lesion diameters were different from each other and stated that virulent and hypovirulent isolates could be separated easily with this method, and also stated that the virulence of the isolates could be measured in a short time.

dsRNA Analysis

The dsRNA analysis of 26 isolates which showed low and high virulence were performed in three recurrences. Positive control and negative control isolates were used. The *CHV1* (USA-2) isolate, which we know that it is a hypovirulent of *C. Parasitica* was used as positive control. As the negative control, the Ki-778, which is a *Leucostoma* spp. isolate was used. Only in 6 isolates were the dsRNA profiles determined; however, in the second recurrence, it was observed that there were dsRNA profile only in 3 of these isolates. Then, in the third recurrence, no dsRNA profiles were determined in any of the isolates, in which dsRNA profile was determined previously (Figure 3). It was determined that the 6 *Leucostoma* spp. isolates in which we determined dsRNA profile were the isolates obtained from the cities of İzmir (Ki-394, Ki-280, Ki-301A, Ki-407), Manisa (Ki-376) and Denizli (Ki-52).

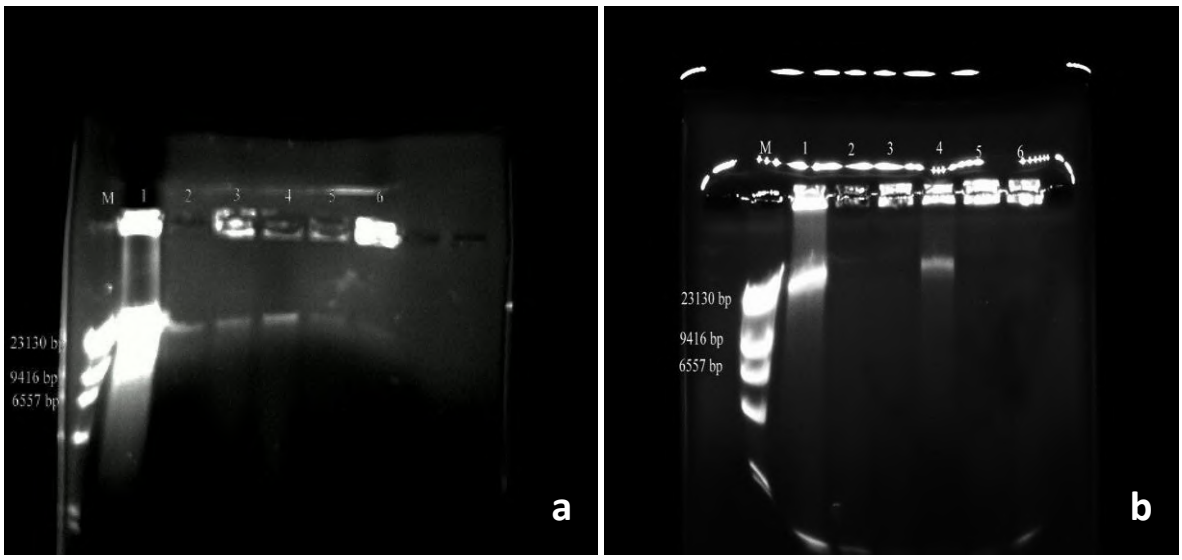

Figure 3. Agarose gel electrophoresis of dsRNA associated with isolate 6 of *Leucostoma* spp.;
a) Marker (M), USA-2 (1), Ki-394 (2), Ki-376 (3), Ki-280 (4), Ki-407 (5), Ki-301A (6)
b) Marker (M), USA-2 (1), Ki-778 pik. (2), Ki-451 (3), Ki-52 (4), Ki-236 (5), Ki-271 (6)

As a matter of fact, Márquez et al. (2007) reported that the dsRNAs, which were found in *Curvularia protuberata* factor were affected by desiccation or freezing- thawing processes and that the dsRNAs were eliminated after these processes. In this study, the reason that there were no bands observed in the recurrent analyses performed with the same isolates, in which

the dsRNA bands were observed, may be due to the reasons like desiccation and freezing-thawing. Peyambari et al. (2014) obtained dsRNA segments whose molecular weights were 1.85, 1.65 and 1.27 kbp from *Cytospora sacchari* isolate obtained from sugar canes in Iran. When they examined the genomic structure of this virus, they determined that it belonged to the *Partitiviridae* family. It was also reported in this study that although mycoviruses had a wide variation, the information on these viruses was insufficient; and that while the structures of some viruses were unstable, some viruses had resistant structures (Peyambari et al., 2014).

Conclusion

The Aegean Region in Turkey has an important place in the economy of the whole country in terms of cherry production. In recent years, the Leucostoma cancer increased seriously in cherry trees in the Aegean Region, and has become one of the complaints of the producers due to the desiccation parts in trees.

In the study, although the dsRNA profile (band) was observed in 6 isolates upon the dsRNA analysis, the dsRNA profiles were not observed in the recurrent isolates. These results, which do not confirm each other, may be explained with the results obtained in the study conducted by Márquez et al. (2007) to eliminate mycovirus from fungus. The structures of some viruses being unstable (Peyambari et al., 2014) make them become vulnerable to desiccation and freezing-thawing. For this reason, repeating this study and determining the isolates of the dsRNA profiles in the structure of fungus may help to find a possibility to fight the relevant factor biologically. However, before this, the effect of the dsRNA on the factor and the genomic structure must be studied. If a hypovirulent relation between the fungus and the virus is determined (just like in *C. parasitica*), the dsRNA, which has been detected with this study, may gain an importance as a biological warfare agent.

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Review paper
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PLUM PRODUCTION AND MARKETING IN TURKEY

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Abstract

Turkey is also the homeland of plum as well as many other fruits, because of the reasons like appropriate climatic and soil conditions, located on immigration routes, existed for living area of many civilizations since the ancient times of history. Being a liked and wanted fruit by consumers along with rich vitamin and mineral content, in terms of colour, taste and flavour is always increased the charm of plum fruit growing. Fruit plum generally exists on markets for a long-period such as 6-7 months. Especially as green plums, they take part an important place among early fruit varieties. Alongside with consumption as fresh fruits, plum is exploited on producing of compote, jam, dried fruits, fruit leather, mash, marmalade, and juice. According to 2012 data of FAO, Turkey grown 2.80% of global plum production which was 10.7 million tonnes, and it was on the 5th greatest grower position after China, Serbia, Romania, and Chile with 300.000 tonnes of plum fruit growing. According to data of Turkish Statistical Institute, Turkey's production of plum fruit was 305.000 tonnes in 2013 and 265.000 tonnes due to drought in 2014. Generally plum fruit growing in Turkey is on the shape of mixed gardens together with other fruits. In this study, some evaluations will be made by revealing the general conditions of plum farming and analyzing of agricultural marketing structure in Turkey. In this study, some researches, articles, presentations, thesis, and statistics were carried out by considering relevant subject.

Keywords: Plum, production, stone fruits, agricultural marketing.

Introduction

Plum is a fruit species, which is known with the latin name "Prunus domestica", belonging to Rosaceae family. According to ancient written documents, it is known that fruits of "Prunus domestica" known for 2000 years have been dispersed from around Caucasia and the Caspian Sea to the world, the homeland of another species named "Prunus institia" is Damascus region (Tunalioglu and Keskin, 2004).

Plum is the leading of mostly consumed fruits among stone fruit species. Its consumption as dried is quite common in the many regions of the world. Thanks to development of food technology, its use in the production of yoghurt, milk, mash, fruit leather, churchkhela, marmalade, jam, chocolate, and dessert and for numerous different products as raw material is gradually increasing as well rather than consuming freshly. One of the effective factors upon the increase in its consumption is that its contribution to health has been revealed as a result of studies conducted. In addition to the phytochemicals of plum and their anti-carcinogenic effects, it is known that plum provides significant benefits for regulation of digestion. Nutritional elements of fruits also make their use essential as mineral supplement as well (Gecer et al., 2015).

It has been determined to inhibitory to chronic diseases especially cancer and hearth disorders besides helping to delay glucose absorption and laxative effects through the phenolic compounds, fibre-rich, fructose, and sorbitol content contained in plum. Furthermore, fruits

consumed as dried are observed to protect cardiovascular health and have a significant role in preventing osteoporosis due to their potassium and boron contents. It is a product which is a must in dietary lists due to such characteristics (Stacewicz-Sapuntzakis et al., 2001).

Plum production in the world was 11.5 million tonnes in 2013. As the Table 1 was examined, it was observed that an increasing trend was obtained even though decreases were seen relatively year by years beginning from 2005. The most important reasons of this increasing trend can be summarised as development of different varieties, increase in number of species and variety adapted to different ecologies, increase of plum production parallel with rise of income level, development of the industry related to this fruit species, increase of its use in food products, and increase of wealth level of population subsisting on this fruit growing along with the increase in number of farmers performing plum production as agricultural activity.

Interest to plum growing is gradually increasing in the world and Turkey due to simplicity of cultural processes in plum growing, quite long harvesting season, and high nutritional values. In addition to high number of sub-species, it is possible to see plum varieties in country market for 5-7 months due to opportunities provided by different ecological regions in Turkey. As the harvest times are considered, it is possible to see plum in the market between 15 April and 15 October (Karamursel, 2011). The first plum harvest begins with green-papaz group belonging to Prunus ceresifera L. specie as early fruits in the Mediterranean region. The final plum harvest ends with late bloomer varieties from Prunus salicina and Prunus domestica group. Marketing period of late bloomer plums can be extended by keeping in different storage conditions regarding species. Marketing period of plum in Turkey extends approximately 9-10 months (Karamursel, 2011).

This study examined and evaluated current condition concerning production, foreign trade, marketing, and consumption of plum in Turkey.

Plum Production in Turkey

Turkey is at the fifth country growing plum in the world after China, Serbia, Romania, and Chile (Table 1). According to data of FAO, net agricultural production value of plum production in Turkey was 182.2 million dollars in 2013.

Table 1. Amounts of plum production in main countries between 2005 and 2013 (Anonymous, 2015a)

Countries	2005	2006	2007	2008	2009	2010	2011	2012	2013
					1000 Tonnes				
World	9.939	10.487	9.621	10.303	10.897	10.719	11.070	10.703	11.528
China	5.200	5.300	4.800	5.200	5.350	5.640	5.850	6.000	6.100
Romania	622	599	373	475	534	625	574	424	512
Serbia	0	556	681	607	663	427	582	391	738
Chile	250	244	250	234	296	298	293	300	306
Turkey	220	214	241	248	246	241	269	297	305
Iran (Islamic Republic of)	166	140	164	269	269	269	289	295	305
United States of America	432	645	367	493	568	257	281	230	210
India	149	160	175	190	191	200	210	215	220
France	214	234	249	161	231	248	177	209	171
Spain	252	179	191	199	233	219	231	205	172
Others	2.434	2.216	2.131	2.227	2.315	2.296	2.315	2.136	2.487

Today, some of plum varieties growing in Turkey are domestic species and some others are foreign species. Domestic species occurred from two species and these were Prunus cerasifera and Prunus domestica species. The mostly grown plum types in Turkey are green plum, papaz plum, damson plum, and sweet thin-skinned plum. Culture varieties derived from Prunus cerasifera species are called "green plums". Major green plum varieties are Aynali, Can-1, Foca, Havran, Karsiyaka, Orta can, and Papaz. Fruits of Can-1, Havran, Orta can, and Papaz varieties are generally consumed freshly and this is a consumption type specific to Turkey (Tunalioglu and Keskin, 2004).

When the balance sheet of plum was examined; it was observed that 305.4 thousand tons of plum were produced in the marketing year covering the period between 1 July 2013 and 30 June 2014, losses occurred during production were 46.7 thousand tons; when import figure of 2.9 thousand tons was added to 258.6 thousand tons of the usable production figure remaining after harvest losses were deducted from production numbers, 261.6 thousand tons of plum were put upon the market (Table 2).

It was observed that 28.1 thousand tons of plum were exported and supplied for foreign consumption in the same marketing year. Therefore, 233.5 thousand tons of products from total production was allocated for domestic use. However, since approximately 18.7 thousand tons were reduced by wastage at processing stage before plum reached to end consumer, domestic consumption of plum in 2013/14 marketing year was 214.8 thousand tons (Table 2).

Table 2. Crop Products Balance Sheets; "Plum" 2005/2006-2013/2014 (Anonymous, 2015b)

	Production	Harvest losses	Supply=Use	Usable production	Imports	Domestic use	Human consumption	Losses	Exports
Marketing year					**Tons**				
2013/'14	305.393	46.725	261.604	258.668	2.936	233.494	214.814	18.680	28.110
2012/'13	300.046	45.907	256.927	254.139	2.788	230.080	211.674	18.406	26.847
2011/'12	268.696	41.110	232.740	227.586	5.154	214.018	196.896	17.121	18.722
2010/'11	240.806	36.843	205.950	203.963	1.987	196.526	180.804	15.722	9.424
2009/'10	245.782	37.605	208.670	208.177	493	198.681	182.787	15.895	9.989
2008/'09	248.736	38.057	210.703	210.679	23	204.010	187.689	16.321	6.693
2007/'08	240.874	36.854	204.744	204.020	724	196.040	180.357	15.683	8.704
2006/'07	214.416	32.806	182.882	181.610	1.272	176.480	162.362	14.118	6.402
2005/'06	220.000	33.660	187.204	186.340	864	173.243	159.384	13.859	13.961

Within the past 10 years between 2005 and 2014, cumulative plum orchard lands reached from 144 thousand decare to 200 thousand decare by increasing at the rate of 39.08%. Production of plum, being 220 thousand tons in 2005, was 265.5 thousand tons in 2014 with the increase of 20.68% compared to 2005 (Table 3). Some factors providing the increase in production can be summarised as follows; different climate conditions of Turkey, development and plantation of species and varieties adaptable to various ecologies, formation of modern orchards, and developments in product processing techniques (Karamursel, 2011; Osmanoglu et al., 2013). When 10-year data found in Table 3 were examined, it was observed that mean plum yield per tree was the lowest with 28 kg in 2006 and the highest level with 37 kg in 2012. Although mean yield per tree was 36 kg in 2013, after a year ie in 2014 mean yield per tree was 31 kg by decreasing the rate of 13.89% (Table 3). The fact that late frost in spring and hail squalls occurred in that year was supposed to be effective upon this decrease. While 86.66% of 8.77 million of total tree number in 2005 were at fruit-bearing age, the number of fruit-bearing trees reached to 8.6 million with an increase of 13.92% in 2014 compared to 2005. According to estimated data of 2014, totally 10.3 million of plum trees were present in cumulative orchard lands of 200 thousand decare. The number of trees at fruit-bearing age in total tree number decreased to 84.07% in 2014 due to establishment of new orchards (Table 3).

Table 3. Plum production amounts in Turkey between 2005 and 2014 (Anonymous, 2015b)

Year	Area of cumulative orchards (decare)	Production(tonne)	Mean yield per tree (kg)	Number of trees at fruit bearing age	Number of fruitless trees	Total number of trees
2005	144.000	220.000	29	7.600.000	1.170.000	8.770.000
2006	140.040	214.416	28	7.571.521	1.150.815	8.722.336
2007	152.545	240.874	31	7.736.690	1.460.626	9.197.316
2008	155.122	248.736	32	7.750.463	1.575.247	9.325.710
2009	166.883	245.782	32	7.743.411	1.571.422	9.314.833
2010	166.244	240.806	31	7.815.844	1.847.120	9.662.964
2011	183.644	268.696	34	7.863.951	1.802.700	9.666.651
2012	193.304	300.046	37	8.187.640	1.794.829	9.982.469
2013	197.262	305.393	36	8.429.484	1.816.936	10.246.420
2014	200.271	265.490	31	8.657.765	1.640.867	10.298.632

*: Data of 2014 are estimated.

According to data of 2014, 31.31% of cumulative plum fields in Turkey are located in the Mediterranean Region, 29.36% in the Aegean Region, and 14.53% in the East Marmara Region. Additionally, 37.81% of plum production performed in Turkey was from Mediterranean Region, 22.16% from Aegean Region, and 16.58% from East Marmara Region (Table 4).

Table 4. Plum production based on agricultural zones (Anonymous, 2015b)

	2013				2014			
IBBS1 name	Area of cumulative orchards (decare)	Production (tonne)	Mean yield per tree (kg)	Total number of trees	Area of cumulative orchards (decare)	Production (tonne)	Mean yield per tree (kg)	Total number of trees
Northeast Anatolia	1.086	3.123	31	141.060	1.097	1.920	19	140.232
Centraleast Anatolia	6.119	10.119	37	328.836	5.717	4.598	18	312.494
Southeast Anatolia	18.667	7.047	25	353.294	17.469	4.623	18	328.175
Istanbul	30	481	23	24.095	30	465	22	23.900
West Marmara	9.891	15.071	28	633.596	9.383	15.693	29	621.119
Aegean	54.319	60.089	32	2.362.132	58.805	58.827	30	2.395.513
East Marmara	28.814	48.354	41	1.323.052	29.109	44.008	37	1.323.622
Western Anatolia	7.574	15.655	41	453.043	7.382	12.851	34	451.657
Mediterranean	62.085	107.535	48	2.807.218	62.702	100.391	42	2.895.727
Central Anatolia	3.949	6.653	25	347.266	4.095	3.257	12	352.267
West Black Sea	3.567	24.323	26	1.116.063	3.533	13.765	15	1.097.059
East Black Sea	1.161	6.943	21	356.765	949	5.092	16	356.867

Plum production is performed in many cities and particularly in Mersin, Hatay and Antalya in Turkey (Table 5). Demand of especially freshly consuming as green plum has increased and this has promoted the development of early grown. Early grown plum production not only provides advantage to consume fresh fruit before its season in terms of consumers but also brings a good income for producers due to high prices.

Table 5. Plum production quantities of some cities in Turkey by 2014 (Anonymous, 2015b)

Order	City	Production Quantity (tonne)	Order	City	Production Quantity (tonne)
1	Mersin	37.579	**11**	Afyonkarahisar	7.191
2	Hatay	21.779	**12**	Denizli	6.709
3	Antalya	17.629	**13**	Kocaeli	6.248
4	Bursa	16.549	**14**	Konya	5.263
5	Adana	15.234	**15**	Karaman	5.227
6	Manisa	11.491	**16**	Balıkesir	4.579
7	Kutahya	11.208	**17**	Isparta	4.227
8	Sakarya	10.004	**18**	Yalova	3.285
9	Izmir	8.512	**19**	Mugla	3.026
10	Canakkale	7.674	**20**	Samsun	2.968

Plum Consumption in Turkey

In Turkey, mean consumption was 214.8 thousand tons and consumption per capita was 2.8 kg according to data of 2013/14 marketing year. According to data of TSI between 2005 and 2014, plum consumption per capita in Turkey varied depending on yield and was about 2.4 kg. While consumption per capita decreased to 2.1 kg in years when the product was scarce, this quantity increased up to 2.8 kg in years when it was abundant (Anonymous, 2015b).

Foreign Trade of Plum in Turkey

As the export data between 2006 and 2015 were examined, it was observed that fresh plum export of Turkey increased gradually. According to data of 2014, 16.1 thousand tons of fresh plum were exported and in return, 11.8 million dollars of foreign currency receipts were obtained (Table 6). It was observed that export of dried plum showed large fluctuations year by year. In 2014, 841 tons of dried plum were exported and in return, 2.5 million dollars of foreign currency receipts were obtained. It could be asserted that import of fresh plum was not performed in large quantities as in export and it differed year by year. It was observed that according to data of 2015; while 12 tons of fresh plum was imported, 8.5 thousand dollars were paid in return of this quantity (Table 6).

According to data of 2006-2013, it could be asserted that even though export unit price of fresh plum changed by years, it had a downward tendency. Thus, it was observed that while export unit price of fresh plum was 1.24 $/kg in 2006, the price decreased to 0.73 $/kg in 2014, mean figure for the first 8 months of 2015 has decreased to 0.48 $/kg. On the other hand, a reverse tendency was observed in export unit price of dried plum. Mean export unit price of dried plum, which was 1.29 $/kg in 2006, reached to 2.99 $/kg in 2014 and increased to 3.45 $/kg based on the mean of 8 months of 2015 (Table 6).

As import unit price of fresh plum was analysed, it was observed that import unit price, like export unit price, was unstable in the same period and showed rise and fall year by year. Importat unit price of dried plum increased as export unit price. While 1.14 $/kg was paid for 1 kg of imported fresh plum in 2014, 2.29 $/kg was paid for 1 kg of imported dried plum (Table 6).

Table 6. Export and import values of plum between 2006 and 2015 (Anonymous, 2015b)

	Export			Import		
Year	Quantity (tons)	Value ($)	Export Unit Price $/kg	Quantity (tons)	Value ($)	Import Unit Price $/kg
			Plum (dried)			
2006	997	1.281.826	1.29	199	260.459	1.31
2007	798	1.652.579	2.07	343	594.944	1.73
2008	589	1.405.995	2.39	81	279.503	3.43
2009	617	927.712	1.50	50	21.613	0.43
2010	559	776.215	1.39	216	386.335	1.79
2011	306	748.521	2.44	1.363	2.248.746	1.65
2012	343	886.392	2.58	730	1.252.049	1.71
2013	586	1.591.893	2.72	803	2.259.963	2.82
2014	841	2.512.252	2.99	750	1.714.420	2.29
2015	217	747.920	3.45	504	1.471.528	2.92
			Plum (fresh)			
2006	2.423	2.998.531	1.24	25	24.419	0.98
2007	3.498	4.181.709	1.20	25	28.104	1.10
2008	3.168	4.194.365	1.32	18	23.142	1.28
2009	6.139	5.268.877	0.86	2	6.622	2.70
2010	7.653	6.086.442	0.80	14	27.185	1.89
2011	11.276	7.605.570	0.67	37	41.001	1.11
2012	25.575	14.157.792	0.55	58	18.195	0.32
2013	24.311	14.752.959	0.61	13	11.561	0.87
2014	16.121	11.785.235	0.73	3	3.075	1.14
2015	24.639	11.849.788	0.48	12	8.502	0.72

*Data of 2014 and 2015 are temporary.

Ten countries to which Turkey mostly exported fresh plum most in 2014 were Iraq, Russian Federation, Bulgaria, Saudi Arabia, Germany, Turkish Republic of Northern Cyprus, Syria, Netherlands, England, and Georgia, respectively. The first ten countries for dried plum exportation were Germany, Belgium, Vietnam, Canada, Bosnia-Herzegovina, Azerbaijan, France, Netherlands, Switzerland, and Austria. While fresh plum was imported only from the Republic of South Africa and Chile in 2014, countries from which dried plum was imported were Serbia, USA, Iran, Kirghizstan, Chile, Morocco, and Moldova, respectively based on the import quantity.

Plum Farmer Take-Home Prices

Farmer take-home prices for plum reformed by producer price index based 2003 reached to the lowest level with 0.62 TL/kg in especially 2012 in last ten years and risen to the highest level with 0.96 TL/kg in 2011. On the other hand, it was observed that there were fluctuations at very low levels year by year at farmer take-home prices and finally mean price of 2014 was 0.83 TL/kg (Figure 1).

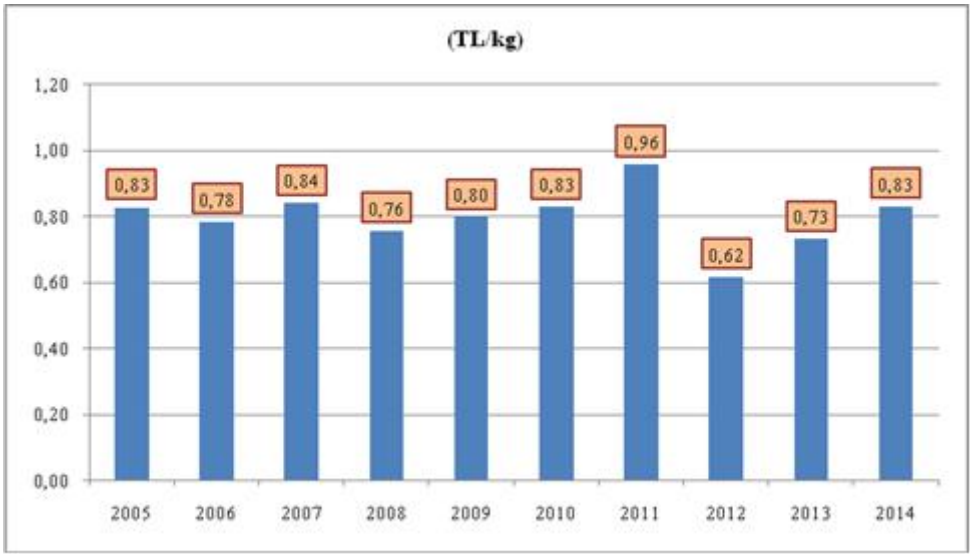

Figure 1. Plum's mean farmer take-home prices in Turkey between 2005 and 2014 (Anonymous, 2015b)

As means of plum farmer take-home prices in ten years by months were examined, it was understood that the price was formed between April and September. According to this, it was observed that while the highest price within the year was in May, the lowest price was in April (Figure 2).

Figure 2. Monthly mean plum prices in terms of farmer take-home prices (TL/kg) (Anonymous, 2015b)

Marketing Channels of Plum

The process in which property of products is passed in other hands and which includes the steps between producer and consumer is called as marketing channel. Structural development of the sector affects the marketing system, as well. Thus, small enterprises with limited marketing opportunities generally dominate in agriculture. This situation cause that enterprises can not sufficiently or never carry out numerous marketing functions such as price formation, standardisation, storage, packaging (Albayrak, 2013). In case that large yield loss

and high expenses are observed because of problems caused by physical processes such as classification, packaging, storage performed for marketing as well excess hand-over of agricultural products until they reach consumer markets and distribution irregularities (Anonymous, 2011). In agricultural product markets; producers, middlemen, brokers, merchants, broker merchants, forwarder merchants, private firms, public institutions, commodity exchanges, wholesale food markets, wholesalers, retailers, cooperatives, and producer unions carry on business (Anonymous, 2011; Albayrak, 2013).

Even though marketing channels for fresh fruits and vegetables in Turkey have showed a configuration focused on actors (middleman, merchant, exporter, producer, etc.) defined basically within the scope of Marketplace Law, different constructions can form based on the product type, target markets (domestic market or export), and regions. Thus, slightly different marketing channels compared to others can be observed in findings of several field studies examining agricultural product marketing and conducted on different products and fields (Sayin et al., 2012). Wholesale food markets are of capital importance trade of fruit and vegetable. According to data of August 2010 from the Ministry of Industry and Trade, there are 203 wholesale fruit and vegetable markets and 9.864 workplaces being still active in these wholesale markets throughout Turkey. In wholesale food markets, producers can both sell their products by themselves and sell the product to brokers in the return of a certain service charge (Canik and Alparslan, 2010).

Conclusion and Recommendations

Plum is a significant fruit type taking place in the third place of the world’s stone fruit production after olive and peach/nectarine by 2013. According to TSI data in 2014, plum production comes after olive, peach, cherry, and apricot production in Turkey and our country takes place among significant plum growing countries of the world with averagely 300 thousand tons of production. Plum is used as table, dried or industrial product; in other words, it can be consumed as raw material in many food products along with its fresh consumption like other several fruit species. In addition to use of plum, having a significant place both in worldwide market and domestic market, for nutrition, value of this fruit type has gradually been increasing as its contributions to health are understood as a result of scientific studies. Improvement of current production and marketing conditions can be realised as a result of using species and varieties appropriate to different ecologies, establishment of protected gardening, choosing correct rootstock, performing regular cultural processes, maintaining plant conservation and nutrition activities, efficiency of harvest and after-harvest processes, market status, and elimination of marketing problems.

As a result of this study, plum production and world ranking status of Turkey was investigated and it was revealed that Turkey can reach a further position thanks to different climate and soil characteristics Turkey has.

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EVALUATION OF ANTIFUNGAL ACTIVITY OF METHANOL PLANT EXTRACTS FROM CORNUS MAS L. AND MORUS ALBA L.

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Abstract

The aim of this study was evaluation of antifungal activity of *Cornus mas* L. and *Morus alba* L. plant extracts against *Rhizoctania solani* and *Botrytis cinerea* under the *in-vitro* conditions. Plant extracts were dissolved with 50% acetone for each doses (10, 50, 100 and 200 mg doses) and added 10 ml PDA (40 ^{o}C). 5 mm diameter fungus mycelium discs were transferred to petri dishes and kept them 7 days for growing fungus mycelium at 25±2ºC in incubator. Fungus mycelium was calculated by compassand compare with control.As a result of the study, 10, 50 and 100 mg doses of plant extracts of *C.mas* and *M. alba* were not inhibited mycelium development of *R. solani* and *B. Cinerea* except 200 mg dose. 200 mg dose plant extracts of *C.mas* was inhibited *R. solani* and *B. cinerea,* 25.60 % and 74.81% respectively. 200 mg dose plant extracts of *M. alba* was inhibited *R. solani* and *B. cinerea,* 60,97% and 80,40 % respectively.

Keywords: C*ornus mas* L., *Morus alba* L., Antifungal

Introduction

Cornelian cherry (*Cornus mas* L) is a species of *Cornaceae* family which drops its leaves in winter and which can grow up to 5-8 meters with a body diameter of 25-45 (Baytop, 1984).*Cornus mas* L., a kind of fruit havinghard cores, is commonly available in our country, especially on coastal regions as well as in mountainous and forestry regions and inside the valleys with a suitable climate (Selcuk and Özrenk, 2011). Mulberry (*Morus spp.*), grown in generally warm and subtropical regions of northern hemisphere, is a species of Morus of *Moraceae* family of Urticales team. Mulberry types which are commonly grown and the fruits of which are derived in our country are *Morus alba* L. (White Mulberry), *M. nigra* (Black Mulberry) and *M. rubra* (Red or Purple Mulberry) as well is its tens of other species (Anonymous, 2015). Usage of natural products became important because of the insufficiency of active substances of synthetic medicines against currently increasing diseases as well as their effects on human health. With this aim, it is ambidextrously researched in terms herbal chemistry, microbiology and pharmacology even as well as in terms of vegetable defense mechanism in the recent years when biological war is a matter of agenda (Kalaycıoğlu and Öner, 1994; Dağcı et al., 2002). This case caused a surprising increase in the number of studies on medical plants which have recently had an antimicrobial activity. Flavonoids having a large number of antimicrobial activities among these plants as well as such chemicals as phenolic compounds, anti-cyanine and tannin protect the plants against infections and wounds (Dildar et al., 2000). It has been known for long years that various herbal extracts have an antimicrobial activity on bacterial and fungal diseases (Dığrak et al., 2002). Lots of herbal extracts have been studied so far throughout the world, but there are few studies regarding *Cornus mas* L. and *Morus alba* L. This study aims at determining antifungal activities of *Cornus mas L.* and *Morus alba L.* against *Rhizoctania solani* and *Botrytis cinerea*.

Material and methods

Plant Materials

The *Cornus mas* L. and *Morusalba*L. Plant materials used in this study have been picked up in August 2014, in Antalya province (Turkey), and dried at room temperature in the shade. They have been stored until being used in trials.

Fungus Cultures

The fungi of plant pathogen used in the study (*Rhizoctania solani* and *Botrytis cinerea*) were obtained from the stock cultures in Phytopathology laboratories of Plant Department of the Faculty of agriculture, University of Ahi Evran.

Preparation of Extracts

For the purpose of obtaining of plant extracts, dried and ground plant materials of 100 g has been located in erlenmeyer of 1 lt, and methanol solvent has been added enough to cover it. It has been subjected to the process of rinsing through a orbital agitator at 120 rpm and room temperature for 24 hours. Then the extract shave been filtered through a filter paper. The solvent shave been kept away by being evaporated at 40 ^{0}C by rotary evaporator. The remaining dry extracts have been dissolved with acetone, and finally, the different concentrations, such as, (1% (10mg) 5% (50 mg), 10% (100 mg) and %20 (200 mg), have been obtained for being used in our study (Kalkisim et al., 2012).

Antifungal effect of Plant methanol extract

Obtained plant extract have been autoclaved in such a way that the last concentrations would be % 1 (10mg), %5 (50mg), %10 (100mg) and %20 (200mg), and then added to the sterile PDA's, which were cooled until 40 ^{0}C. The PDA's have been transferred to 60 mm diameter petri vessels (in away as being 10 mm). The mycelium discs taken from 7-days fungus cultures previously have been transferred to 5 mm diameter petri vessels. After inoculation, fungus cultures have been subjected to incubation at 25±2 0C for 7 days, and at the end of the period, developed radial mycelium have been taken (Hadizadeh et al., 2009). The development of mycelium has been calculated according to the formula given below (Pandey et al.,1982). As positive control, a standard fungicide has been used containing %50 captan. Thetrial was conducted in through four repetition sand two iterations

$$MGI=100\times(dc-dt)/dc$$

MGI:percentage mycelial growt hinhibition

dc; mycelia growth in control

dt; mycelia growth in treatment

Results and Discussion

The effect of *Cornus mas* L. and *Morusalba* L. methanol extract on *Rhizoctonia solani* and *Botrytis cinerea* mycelium development is displayed in (Table 1) .

Table 1. Effects on plant pathogenic fungi mycelium growth of *Cornus mas* L. and *Morus alba* L. methanol extracts (mm).

Application dose	*Cornus mas* L.		*Morus alba* L.	
	***R. solani* mycelium growth (mm)**	***B. cinerea* mycelium growth (mm)**	***R. solani* mycelium growth (mm)**	***B. cinerea* mycelium growth (mm)**
Positive control	0,00c*	0,00c	0,00c	0,00c
Negtative control	60,00a	60,00a	60,00a	60,00a
10mg	60,00a	60,00a	60,00a	60,00a
50mg	60,00a	60,00a	60,00a	60,00a
100mg	60,00a	60,00a	60,00a	60,00a
200mg	44,60b	15,11b	23,41b	11,76b

*According to DUNCAN, The averages with different letters at the same column are different at $p<0.05$ significance level

Although there are differences in the effect depending on the dose of applied herbal extract and diseases, it has been determined that *Cornus mas* L. and *Morus alba* L. methanol extract generally has an activity. The highest dose of *Cornus mas* L. methanol extract prevents the development *Rhizoctonia solani* and *Botrytis cinerea* mycelium development respectively in the rates of 25,6 % and 74,81 %. On the other hand; the highest dose of *Morus alba* L. methanol extract prevents the development of *Rhizoctonia solani* and *Botrytis cinerea* mycelium development respectively in the rates of 60,97 % and 80,40 % (Figure 1) .

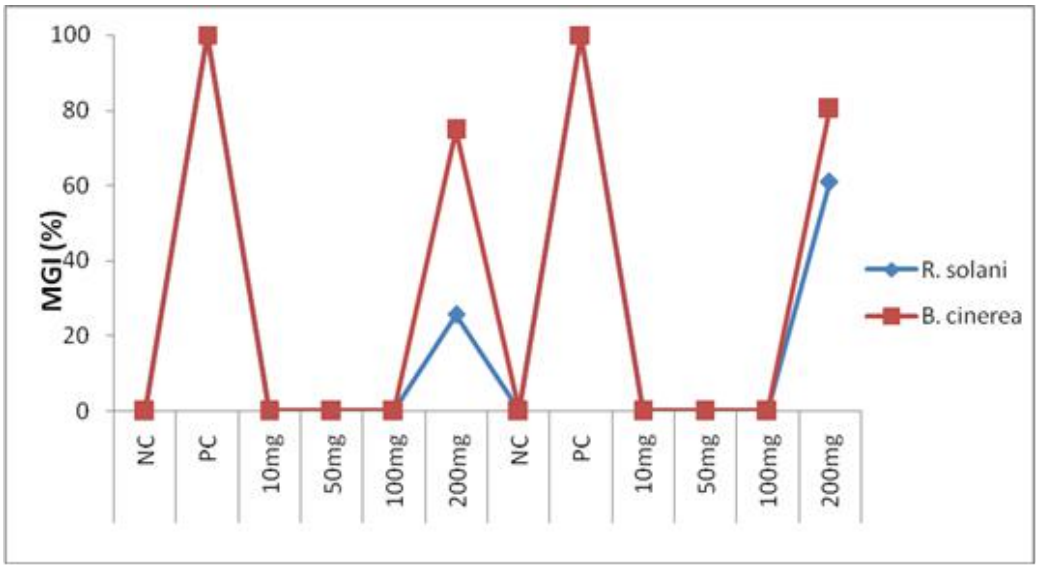

Figure 1. The inhibition ratio of methanol extract of *Cornus mas* L. and *Morus alba* L. on the development of mycelium of plant pathogen fungi.

Cornus mas L. and *Moru salba* L. plants arise out of such compounds as phenolic and flavonoids which are active compounds having biological activity within themselves (Danica et al., 2014; Pawlowska et al., 2010). It has been determined that *Morus alba* L. methanol and water extracts have antifungal and antibacterial activities (Jha and Srivastava, 2013). On the

other hand; *Cornus mas* L. has a high level of antimicrobial activity (Anthony and Dinda., 2015).

Conclusions

This study was made of *Cornus mas* L. and *Morus Alba* L. The methanol extracts to determine the anti-fungal activity. *Cornus mas* L. 200 mg dose of study results *Rhizoctonia solani* and *Botrytis cinerea* mycelium development in the rates of respectively 25,6% and 74,81%. *Rhizoctonia solani* and *Botrytis cinerea* study results *Morus Alba* L. 200 mg dose mycelium development respectively in the rates of 60,97 % and 80,40 %. Our results show that methanol extracts of *Cornus mas* L. and *Morus alba* L. plants have antifungal activities against *Rhizoctonia solani* and *Botrytis cinerea*.

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THE IMPACT OF FLOODS ON THE CHANGE OF PHYSICAL AND CHEMICAL PROPERTIES OF AGRICULTURAL SOIL

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Abstract

According to the estimates, after last year's floods the main damage is reflected in the loss of return on agricultural crops. Agricultural cultures have long been under water. With the arrival of the spring period, it was found that significantly more damage done to the land, and in order to improve the quality and restore the land to the level before the floods it will be necessary to invest substantial funds for a longer period.

Depending on the length of the impact of floods on land depends the level of changes in physical and chemical properties of the soil. Land that was under water for a short time has expressed: pH change , change of compaction of soil and nutrient content.

During the spring a higher percentage of weed plants was found and poor effective herbicides as well. In areas where water was retained longer period the increased concentration of heavy metals was determined. For quality of sowing soil preparation is necessary to apply a much more scientific farming operations. Some operations have had to be repeated several times to land led to a favorable condition for planting. In respect of land which was not flooded, for the treatment of flooded land consumption of fuel, lubricants and time increased by 30 to 50%. When processing flooded soil abrasion machines for processing higher by 30%.

Agronomists should give a recommendation and clear instructions that measures should be implemented to restore the quality of the soil.

Keywords: *land, flood damage, pH, compaction, weeds.*

Introduction

The soil and especially the soils that are used in an intensive agricultural production are exposed to various degradation processes. The soil limits the fulfillment of goal, increased food production, because every day there is a reduction in surfaces and quality of agricultural soil. The soil quality is defined as the ability of soil to perform its functions within the limits of the ecosystem, maintaining the biological activity too, and providing the environment for plants and animals (*Doran et.al., 1994*). It is considered that the notion of soil quality includes physical, chemical and biological properties. In the long run, the high quality soils ensure the productivity of plants with a minimal impact on the environment (*Baere et al., 1997*). The soil fertility is closely related to the soil quality, and represents the ability of soil to ensure a satisfactory crop production with the minimal use of fertilizers and manure (*Hansen et al., 2001*). In addition to the direct negative effect of heavy metals on human health, they also disturb the delicate balance in the ecosystem and negatively affect the living world, which indirectly endangers man’s position in biogeocenosis for a longer time period (*Bennett et al., 2001*). About 135 million tons of phosphate ores is produced yearly around the world, which leads us to conclusion that there is a constant danger of intake of around 21000 tons of uranium in the soil through mineral fertilizers (*Dangić, 1995*). The production capacity of soil

is reduced by processes of continuous reduction and damage to agricultural areas. The floods that during 2014 affected the region of former Yugoslavia caused enormous harm to agricultural soil. At the territory of BiH, the floods caused a series of direct and indirect damages to the soil.

Materials and Methods

The investigations were carried out during the spring in 2015 at the territory of Semberija region (Republic of Srpska, Bosnia and Herzegovina) that has suffered the damages caused by floods. The soil sampling has been performed at the flooded territories for determining the changes in pH value of soil, the contents of humus and easily available phosphorus. This part of investigations was performed during February and March, while the second part of investigations was continued during March, April and May. In this part of investigations the condition of agricultural soil was analyzed and the level of soil degradation caused by flood was being determined. During soil cultivation, it was analyzed how much time it was needed for soil preparation, which agro technical operations were included, and how much material and fuel was spent. Weediness and effectiveness of herbicides was being monitored on the production plots.

Results and Discussion

During the investigation, a special emphasis was put on the division of the areas according to the duration of retention of flood wave at the flooded area as the assumption was that the duration of the retention of water on the agricultural soil was in correlative relation with the damages, i.e. the degree of soil degradation. The results show that there is no firm relationship between the stated values and that they cannot be related in any respect. Of all collected samples, the largest number had the changes in pH value which ranged in an interval from 5.7 to 6.5. Compared to the previous state of pH value, which ranged in an interval from 6.8 to 7.2, it is clear that there was a transition of pH value of soil into the class of acid to slightly acid soils which was influenced by flood. The change in pH value of soil can be related to the influence of a number of factors of which the most important are the flushing and loss of carbonate complex, flushing of humus layer, loss of the organic matter and movement of nutrients into the deeper layers.

The analysis of the humus content in the soil confirms the indications, since a medium to low humus content was determined in all soil samples. The humus content ranged from 1.5 to 2% (20 samples) and in an interval from 2 to 2.5% (ten samples). It is clear that the effect of flood is obviously evident on the humus content in the soil because under the influence of water there is a loss of humic matter in the soil. We came to the same results by analyzing the content of easily available phosphorus and potassium, where the determined values ranged from 8 mg P_2O_5/100g and 8 mg K_2O/100g of soil up to 15 mg. Compared to the previous condition of soils that were well provided with phosphorus and potassium (with a content of over 20mg/100g), we now determined a significant loss of nutrients.

The results of the analysis match the indications obtained in an investigation by *Kljajić et al. (2012).* The duration of flood wave, which retained on the plots for longer than five days, caused irreversible losses of nutrients.

During the vegetation period, the analyzed plots were monitored where we tried to determine the visual condition of crops. The general conclusion is that all the plots had very bad visual image. It was reflected in unequal development of crops where ’waves’ are noticeable i.e. much emphasized symptoms of lack of nutrients. Small, underdeveloped and stunted plants with an emphasized yellow-chlorotic color dominated on the plots of Semberija. This phenomenon was emphasized not only in the flooded area but also on all the plots that had been under the influence of long-term rainfalls, and therefore intensive flushing. According to

Vučić (1992), it is difficult to separately observe the effect of various chemical agents that are being applied in agriculture only on soil without the wider reflection on nature and the environment. When it comes to the pollution of soil the problems should not be solved but prevented. There are a lot of the polluters of soil, they come from different parts, they are not equally distributed, all of which complicates consideration of the degree of qualitative and quantitative level of pollution and proposal of measures for its repairs.

Very great damages to the agricultural soil are reflected through the loss of soil structure. Good soil structure is a good basis for the increased soil fertility because one of the basic conditions is fulfilled for the intensive growth of the roots of the plants, which is a good water-air regime. Under the influence of water, which had been laying on the soil for days, structural aggregates compact, fine porous structure of the soil is lost whereby macro and micro aggregates are completely lost. The changes in soil structure cause the changes in air regime where there is obvious lack of oxygen for root respiration, microbiological activity is slowed and the possibility for accumulation, storage and supply of water is minimal. During the vegetation, there is a constant problem which is connected with the increased compactness of soil, which is that only the surface layer of soil has the ability of accumulating the rainfalls, and which leads to a constant occurrence of excess or lack of humidity.

Unstructured soils are unfavorable and difficult to cultivate because it is necessary to use more agro-technical operations in order to properly prepare the soil for planting. The compacted soil is more difficult to cultivate and our investigation moved in that direction. We wanted to come to an answer how much more fuel, tools and time is spent for soil cultivation compared to the period before the flood.

The fuel consumption was highest in plowing and it ranged up to 25 l/ha, which, compared to the consumption in the cultivation of the soil that was not flooded, means the increase of 40 to 50%.

For disking, the fuel consumption was increased for about 35% (disking in two passes) while for the harrowing the fuel consumption increased for about 30%.

All of these agro-technical measures could not adequately respond to the task, i.e. to properly prepare the soil for sowing but the preparation of soil was mostly combined with the use of power harrow and seed bed tiller. The fuel consumption when using the power harrow was increased for 35%, and for 25% when using seed bed tiller, compared to the cultivation of soil that was not flooded. The increased compactness of soil has led to the increase in fuel and time consumption, and for the preparation of 1 ha of soil, the time of preparation increased for 30 to 40%, on average.

The change in structure and other properties of soil should lead to the change, i.e. harmonization of the applied agro-technical measures, so that better effect i.e. better soil preparation would be achieved with the minimal fuel and time consumption.

By monitoring the parameters of certain working operations, we came to the conclusion that it is necessary to include undermining at the depth of 30 to 40 cm within soil preparation. By including of the afore-said measures within the cultivation of soil we come to conclusion that with such tools for soil cultivation soil preparation is far better and economic where it is evident that small crumbly fractions of soil dominate in the seeding layer.

After undermining, the plowing is lot easier and it is done with a reduced consumption of fuel and the total fuel consumption for undermining and plowing is 28 L/ha, which is by three l more than in the case of the single measure of plowing, and the effect is much more greater.

Under the influence of the flood, during the autumn and spring period, a significantly higher occurrence of weed was recorded. On the soil that was not treated from 370 to 470 of weed seedlings per m^2 was recorded. The largest number of weed plants belongs to:

1. Ambrosia artemisiifolia-ragweed,

2. Omenopodium sp.-meldweed,
3. Convolvulus arvensis-field bindweed,
4. Galium sp.-hedge bedstraw,
5. Sinapsis arvensis-field mustard,
6. Abutilon theophrasti-abutilon,
7. Amaranthus retroflexus-amaranth and
8. Datura stramonium-jimson weed.

During the May in 2014, the dominant weed species were not in the phase of the formed seed, but it was most often the vegetative phase. Such a mass phenomenon of weeds can be explained only by raising the seeds from the lower layers to the surface layer of soil. Another logical explanation can be linked to the application of an enormous number of seeds of various weed species from the upper streams of rivers so that brought seeds would deposit in the plains of Semberija.

After the sowing of corn, the effectiveness of herbicides on the occurrence of weed plants in crops was analyzed. The analysis included 60 plots which differed in soil type, but the plots that were treated with the same combination of herbicides were selected. The treatment of spraying was carried out after the sowing and before seedling emergence of maize. The satisfactory effectiveness of herbicides was recorded on 10 out of 60 plots of maize. The herbicides did not have the satisfactory effectiveness on the remaining 50 plots because even after the treatment the emergence of more than 80 saplings of weed plants per m^2 was recorded. The analyzed plots were sampled cross diagonally with the distance of sampled square of 20 meters. The low effectiveness of herbicides can be attributed to the extremely high temperatures during the treatment, the change in pH value of soil, reduction of humus and organic matter in the soil, change in the soil structure, and a very high abundance of weeds.

Conclusion

There was a considerable change in physico-chemical properties of soil on all the flooded areas in Semberija. The analysis of samples confirmed that the degradation effect of flood caused the change in pH value of soil. The effect of the flood is reflected in an intensive flush and loss of humus, too, as well as other nutritive macro elements.

The intensive occurrence of weed plants, as well as weak effectiveness of the herbicides, is only one of the negative effects of the flood that is subsequently reflected. The floods have drastically changed the physical properties of soil which is reflected in the change of the structure i.e. the compactness of soil. After the flood, it is very difficult to cultivate the soil well, and that leads to significantly higher fuel and time consumption and a higher amortization of machinery. A comprehensive analysis of soil should provide a clear guidance on how to prevent the further loss of soil quality and what is necessary to apply in order to improve the soil, i.e. to bring the soil back to the state of quality it had before the floods.

Proper use of machinery for cultivation of soil, a constant control of soil fertility and a rational use of limestone, organic and mineral fertilizers, as well as cultivation of crops that lead to the improvement and protection of soil must be the first steps in the improvement of soil quality.

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EFFECT OF VARIETY AND SIZE OF PLANTING MATERIAL TO POTATO TUBERS YIELD

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Abstract

The paper presents the results of two-year study of the impact of the variety and size of planting material of potato in the area of Lukavica (East Sarajevo). In years of research, the highest yield in the experiments was found in the variety Faluka, while the lowest yield was found in the variety Agria and in the variety Aladin in 2013 and 2014 respectively. The largest number of tubers in all studied years was found in the variety Aladin (in 2013 – 12.30 and in 2014 – 10.55), while the lowest number of tubers was found in the variety Agria (in 2013 -8.25 and in 2014-8.14). Tubers with a maximum mass were obtained at variety Faluka (121.25 g in 2013 and 101.44 g in 2014), while the smallest tubers were found in the variety Aladin (103.75 g in 2013 and 90.81 g in 2014).

In situations where we planted larger tubers per plant, we got a larger number of tubers (by planting tubers weight of 40 g we got 9.29 tubers in 2013 and 7.42 tubers in 2014, and by planting tubers weight of 60 g we got 10.89 tubers in 2013 and 9.46 tubers in 2014), but their weight was lower compared with alternatives where we planted smaller tubers. By planting small tubers we got yield of 50.24 t ha^{-1} and 42.86 t ha^{-1} in 2013 and 2014 respectively, and by planting large tubers we got yield of 52.43 t ha^{-1} and 44.97 t ha^{-1} in 2013 and 2014 respectively.

During the growing season (April-August) the average daily temperatures were 17/26 ° C, and 16/10 ° C in 2013 and 2014 respectively, while the total amount of rainfall during the growing season in 2013 was 359.5 mm and 610 mm in 2014. Adverse conditions in the early stages of growth and development of potatoes in 2014 due to excessive rainfall affected the number of formed tubers, tuber weight and yield. In 2013 the weather conditions were more favorable, with the exception of July where there were deficit of rainfall (27.3 mm), but due to the adequate application of agro-technical measures the lack of rainfall in July had not affected the yield of potato tubers negatively.

Key words: *variety, potato, tuber number, tuber size, weight, yield.*

Introduction

Potato production in Bosnia and Herzegovina is lagging behind potato production in developed countries. The average yield of about 11 t ha^{-1} puts us at the bottom of the European potato producers. Such low yields are mainly due to the use of low-quality seedlings, poor cultivation practices, production without irrigation, traditionally superficial approach towards production (Đorđević, 2000a and b; Ćota et. al., 2000; Milić and Bogdanović, 2009; Milić et.al, 2012). Production is often carried out in conditions of extremely unfavorable impact of environmental factors, mainly high temperatures and precipitation deficit during pollination and grain filling tubers. Inadequate water-air regime of the soil and high air temperatures usually occur in June and July, at the time of intensive growth and development of potato respectively. Whit the action of unfavorable external factors, extensive and inadequate land cultivation and crop care cannot provide optimal conditions for the growth and development of potato plants.

By selecting appropriate varieties, optimal planting deadline, proper selection of the size of planting tubers and appropriate agricultural techniques adverse impact of external factors can be overcome, because some varieties, with the faster growth, good coverage of soil, and early seeding of tubers, pass the critical stages of development in favorable conditions (Jovović et al., 2012; Poštić et. al., 2012a; Poštić et. al., 2012b; Dardić and Dimitrić, 2009). By knowing the production potential of varieties and choosing certain factions for planting we can influence on increase in the productivity of the crop - yield and tuber, yield structure, tubers and better technological and nutritional quality.
The aim of this study was to determine, on the basis of field trials, the optimal size of tubers as planting material and the potential for yields of selected varieties.

Material and method

The study of genetic yield potential of some potato varieties (factor A - Agria, Aladin and Faluka) depending on mass planting tubers (factor B - tubers weight 40 g or tuber weight 60 g) was performed in the low lime soil (table 1), at an altitude of 550 meters in Lukavica (East Sarajevo) in 2013 and 2014 (factor C-year). The field experiment was conducted in a randomized block design, in 4 replicates and plot size was $30m^2$. Primary treatment, preparation and soil fertilization were performed in a way standard crop of potatoes calls for. The protection of crops from weeds, pests and disease-causing agents were conducted in a timely manner.
Determination of the average number of tubers and the average mass of tubers ere performed at each iteration of a sample of 10 homes after maturation. The total yield per hectare was made by theoretical categories for crop density (about 48000 plants per hectare).
Statistical analysis was done by the method of factorial analysis of variance (ANOVA), using the statistical package Statistics 5.5 (Windows, analytical software), and the score differences between means was performed using LSD test.

Table 1. Chemical characteristics of acid-brown soil on experiment field

Depth (cm)	pH_{H_2O}	Humus	N	Soluble mg/100 g	
		%	%	P_2O_5	K_2O
0-30	6.63	3.62	0.23	14.75	15.59
30-50	6.95	2.15	0.14	2.50	9.29

Meteorological data during performing experiments are shown in table 2.

Table 2. Meteorological conditions during the experiment

Year	Month					Average
	April	May	June	July	August	
Air temperature(0C)						
2013.	12.4	15.2	18.3	20.5	19.9	17.26
2014.	10.2	13.5	17.6	19.5	19.7	16.10
Amount of rainfall (mm)						Total
2013.	64.2	136.0	63.5	27.3	68.5	359.5
2014.	148.0	186.0	125.0	73.0	78.0	610.0

Research results and discussion

The number of formed tubers, as well as the uniformity of their size, depends on not just the inherited characteristics of the variety, but also on external influences. In years with normal climatic conditions tuber formation begins 4-5 weeks after sprouting. If, during this period, moisture is sufficient tubers will be formed at all stolons. In the absence of moisture during this period the plant reduces the number of stolons and forms a small number of tubers. The measurement results (tab. 3) shows that the largest number of tubers in all the studied years, was found in the variety Aladin (12.30 and 10.55 in 2013 and 2014 respectively) while the lowest number of tubers was found in the variety Agria (8.25 and 8.14 in 2013 and 2014 respectively). The variety Aladin, in comparison with the varieties Agria and Faluka, had a highly significant greater number of tubers. The total number of tubers grows when planting bigger seed potatoes, and planting them at longer row spacing leads to formation of large tubers (Broćić et al., 2000). To achieve high and stable yields the best varieties are those that stirs about 10 tubers per plant (Bugarčić et al., 2000). Analysis of the average number of tubers per plant, depending on the size of planted tubers, for tubers of 40 g gave the following results (9.29 and 7.42 in 2013 and 2014 respectively), and for tuber weight of 60 g (10.89 and 9.46 in 2013 and 2014 respectively). By planting larger tubers we got a statistically significantly greater number of tubers per plant compared with the number of tubers in the trials where we planted smaller tubers.

Table 3. Number of potato tubers per plant

Tuber weight / Variety	2013		Average	2014		Average	Two-year average
	I	II		I	II		
Agria	7.62	8.87	8.25	7.10	8.95	8.03	8.14
Aladin	11.35	13.25	12.30	7.70	9.92	8.81	10.55
Faluka	8.90	10.55	9.70	7.74	9.50	8.48	9.09
Average	9.29	10.89	10.08	7.42	9.46	8.44	9.26

LSD	A	B	C	AxB	AxC	BxC	AxBxC
0.05	0.621	0.507	0.507	0.769	0.769	0.717	1.243
0.01	0.778	0.675	0.675	1.025	1.025	0.965	1.657

The average number of tubers regardless of variety, tuber size and year was 9.26. In 2013, the average number of tubers per house was 10.08 and, in comparison with 2014 (8.44), it was statistically significantly higher. Larger number of tubers in 2013 was due to weather conditions that were favorable compared to 2014 (table 2). Average monthly temperatures in April and May in 2013 that were higher compared to 2014, resulted in the faster and more uniform germination and sprouting of potatoes, forming stolons and the number of tubers. A large number of rainy days, as well as large amounts of rainfall in April and May in 2014 adversely affected the growth and development of potato. Due to unfavorable weather conditions there were delays in the implementation of some agro-technical measures. Frequent shifts of rainy and warm days influenced the occurrence of disease.

Size of the tubers is varietal characteristics, but it also depends on the level of agricultural measures, type of soil and its structures, as well as on the meteorological conditions during the vegetation season (Ilin et al., 1992). Availability of water from early formation to maturity of tubers of plants is is very important for the manifestation of hereditary characteristics of a variety in terms of uniformity of tuber size and height of the total yield (Suvajdžić and Šilić, 1984).

Tubers with the greatest mass were obtained in variety Faluka (121.25 g and 101.44 g in 2013 and 2014 respectively), while the variety Aladin had the smallest tubers (103.75 g and 90.81g in 2013 and 2014 respectively). Analysis of variance of the average weight of tubers showed a statistically significant increase in mass of the cultivar Faluka compared with all other varieties in 2013, whereas the difference in 2014 was in comparison with the variety Aladin.

Size of tubers is conditioned by number of canes that are formed by a single plant, and if the number of primary canes is higher the number of tubers formed is higher, but not their masses and vice versa (Bugarčić, 2000 b; Butorac and Bolf, 2000; Jovović, 2002; Jovović et. al, 2010). These results are in line with our results. In cases where we planted larger tubers per plant, we got a larger number of tubers, but their weight was lower compared with alternatives where we planted smaller tubers. This difference in 2013 (average weight of tubers at planting smaller tubers of 117.5 g, and average weight of tubers at planting larger tubers 102.5 g) was significantly higher, while in 2014 that difference had not statistical significance (average weight of tubers at planting smaller tubers was 99.17 g and average weight of tubers at planting larger tubers was 93.58 g).

Table 4. The average mass of potato tubers in g

Tuber weight / Variety	2013		Average	2014		Average	Two-year average
	I	II		I	II		
Agria	95.00	115.00	105.00	103,50	90.25	96.87	100.95
Aladin	120.00	87.50	103.75	85.63	96.00	90.81	97.28
Faluka	137.50	105.00	121.25	108.38	94.50	101.44	111.34
Average	117.50	102.50	110.00	99.17	93.58	96.37	103.18

LSD	A	B	C	AxB	AxC	BxC	AxBxC
0.05	7.205	5.957	5.957	10.318	10.318	8.425	14.592
0.01	9.727	7.942	7.942	13.756	13.756	11.233	19.456

The average mass of tubers regardless of variety, tuber size and year was 103.18 g. In 2013, average mass of tubers 110 g and compared to 2014 (96.37 g) it was significantly higher.

Achieving high yields of potatoes is conditioned by a number of factors. In addition to favorable climatic conditions during the vegetation season the other factors are: the variety, adequate amounts of fertilizer and application of appropriate tillage soil system. In both years the highest yields were found in the variety Faluka (53.92 t ha^{-1} and 47.15 t ha^{-1} in 2013 and 2014 respectively). The varieties and Aladin had the lowest yield (Agria-46.35 t ha^{-1} and Aladin-41.74 t ha^{-1}) in 2013 and 2014 respectively. In 2013, the variety Aladin and Faluka, in comparison with the variety Agria, had a statistically significantly higher yield, while in 2014 the variety Faluka had a statistically significantly higher yield compared to varieties Aladin and Agria.

Size of planting material is an important factor for successful potato production. Size of tubers is important for the initial pace of growth when the plant lives at the expense of spare nutrients from stem tubers. Soon after the release of root the initial effect of size is lost. This is an important fact to keep in mind, with regard to the participation of planting material in the total cost of potatoes production (Ilin et. al, 2000). Đorđević and Jovanović (1994) achieved the lowest yield during planting tubers weighting of 35-40 grams. By planting the tubers weighting 75-80 grams, the yield was higher than the first combination for 20,46%. Based on examination results regarding influence of planting tubers size on potato yield Jakovljević and Šušić (1965) concluded that the small tubers (30-40 g) give lower yields compared to the larger tubers (70-80 g). The results obtained in this study are consistent with results of previous authors because yield after planting small tubers was 50.24 t $ha^{-1}$and 42.86 t ha^{-1} in

2013 and 2014 respectively, while yield after planting larger tubers was 52.43 t ha^{-1} and 44.97 t ha^{-1} in 2013 and 2014 respectively. These differences were statistically significant.

Table 5. The yield of potato (t ha^{-1})

Tuber weight / Variety	2013		Average	2014		Average	Two-year average
	I	II		I	II		
Agria	45.25	47.45	46.35	42.71	43.00	42.85	44.60
Aladin	52.70	54.77	53.73	41.13	42.35	41.74	47.74
Faluka	52.77	55.07	53.92	44.75	49.56	47.15	50.54
Average	50.24	52.43	51.33	42.86	44.97	43.91	47.62

LSD	A	B	C	AxB	AxC	BxC	AxBxC
0.05	1.975	1.613	1.613	2.794	2.794	2.281	3.951
0.01	2.633	2.150	2.150	3.323	3.323	3.041	5.265

The average yield regardless of variety, tuber size and year was 47.62 t ha^{-1}. In 2013, average yield was 51.33 t ha^{-1} while in 2014 it was 43.91 t ha^{-1}. Difference in yield in two years was statistically significant.

Conclusion

Based on the results of two years of research about the influence of the variety and tuber size on the productive characteristics of the potato in field trials conducted in Lukavica, the following conclusions can be deduced:

According to the analysis of climate and soil data, conditions for growing potatoes as the main crop, planting in the period from the beginning of the third decade of April, in the area of Lukavica, were relatively favorable. By timely planting of potatoes in this area physiological activities of plants with favorable natural conditions of the area can be matched and thus high and stable yields can be achieved.

The number of formed tubers, as well as uniformity in size, depends on the hereditary characteristics of the variety, as well as from external influences. Variety Aladin had the highest and variety Agria the lowest number of formed tubers per plant. The total number of tubers formed is higher by planting large tubers.

Size of the tubers is varietal characteristics, but it also depends on the level of agricultural measures, type of soil and its structures, as well as on the meteorological conditions during the vegetation season. The variety Faluka had the largest tubers while the variety Aladin had the smallest ones.

Achieving high yields of potatoes is conditioned by a number of factors. In addition to favorable climatic conditions during the vegetation season the other factors are: the variety, adequate amounts of fertilizer and application of appropriate tillage soil system. In both years the highest yields were found in the variety Faluka, while the variety Aladin, due to various meteorological conditions, proved to be unstable variety.

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VARIABILITY OF QUALITATIVE CHARACTERISTICS AND YIELD OF SOME POTATO VARIETIES IN THE AREA OF SARAJEVO-ROMANIJA REGION

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Abstract

We examined the productive characteristics of some potato varieties (yield and commercial yield of potatoes) in agro-ecological conditions of Sarajevo-Romanija region during 2013 and 2014. Regarding the qualitative characteristics we examined the content of dry matter and starch. Varieties showed variability in yield and commercial yield of tubers. The greatest variability was found in a variety Aladin which in 2013 and 2014 had a yield of 52.90 t $ha^{-1}$and 40.25 t ha^{-1} respectively. The smallest variation in yield was found in the variety Desiree 36.72 t ha^{-1} and 34.40 t ha^{-1} in 2013 and in 2014 respectively. The variety Aladin had the highest yield of marketable potato. The variety Agria is suitable for processing into French fries and potato chips. This variety had the highest dry matter content, which ranged from 21.27 % to 22.50%, while the variety Kondor had the highest starch content (from 18.22% to 18.97%). In 2013, due to favorable weather conditions, we achieved better results both in productive and in the qualitative characteristics of the potato.

Key words: *potatoes, variety, productive characteristics, quality, yield.*

Introduction

For achieving high and stable potato yields, apart from favourable climate factors during the vegetation period, key factors are also potato variety, application of adequate amounts of mineral fertilizers and application of appropriate cultivation systems and plant care (Jovović, 2010; Milić et Bogdanović, 2009; Milić et al., 2012). For better usage of genetic potential of the potato varieties it is necessary to examine productive and qualitative characteristics of potatos in different agroecological environmental conditions (Bugarčić i sar., 1991: Đukić i sar., 2000). Except for achieving high and stable potato yield it is important to achieve high yield of mercantile tubers-commercial yield (Broćić et al, 2000) as well as select varieties meeting technological characteristics of potato for production of potato chips and French fries (Jackson et Berga, 2003; Henryk, 2012).

The aim of this study is to select varieties which, based on field experiments, produce high yields of mercantile potatoes-commercial yield and meet needs of food industry regarding the quality.

Material and method

The study of genetic yield potential of some potato varieties was conducted in Lukavica (East Sarajevo) in 2013 and 2014, on the slightly lime soil (table 1), at an altitude of 550 m. The following potato varieties with different vegetation period and skin color were tested: Agria, Aladdin, Faluka, Kondor and Desiree. The field experiment was conducted in a randomized block design, in 4 repetitions with basic plot of $15m^2$.

Basic processing, preparation of the soil and fertilization were carried out as standard procedure for potatoes. Weed control, treatment of causes of diseases and pests were carried out in a timely manner.

Digging of the physiological mature tubers determined the total yield while the mercantile/commercial potato yield is determined by taking healthy, properly developed tubers weighing more than 70 g.
The content of starch in the tubers was determined by Ewers, and dry matter content was determined by drying at 105^{o}C.
Statistical analysis was done by the method of factorial analysis of variance (ANOVA), using the statistical package Statistics 5.5 (Windows, analytical software), and the difference between means was performed using LSD test.

Table 1. Chemical characteristics of acid-brown soil on experiment field

Depth (cm)	pH_{H_2O}	Humus	N	Soluble mg/100 g	
		%	%	P_2O_5	K_2O
0-30	6.63	3.62	0.23	14.75	15.59
30-50	6.95	2.15	0.14	2.50	9.29

Meteorological data during performing experiments are shown in table 2.

Table 2. Meteorological conditions during the experiment

Year	Month					Average
	April	May	Jun	July	August	
Air temperature(0C)						
2013.	12.4	15.2	18.3	20.5	19.9	17.26
2014.	10.2	13.5	17.6	19.5	19.7	16.10
Amount of rainfall (mm)						Total
2013.	64.2	136.0	63.5	27.3	68.5	359.5
2014.	148.0	186.0	125.0	73.0	78.0	610.0

Research results and discussion

In the two years examination the average yield of potato tubers was 43.39 t ha^{-1}. In 2013, the variety Aladin had the highest average score (52.90 t ha^{-1}), while the variety Dezire had the lowest one (36.72 t ha^{-1}). Estimated differences were statistically significant. The varieties Agria, Faluka and Kondor in comparison with the variety Desiree had statistically higher yield, while the yield differences between these varieties did not have statistical significance. In 2014 the variety Kondor had the highest average score (43.12 t ha^{-1}), while the variety Dezire had the lowest one (34.40 t ha^{-1}). The differences were not statistically highly significant, and the differences between the other varieties in comparison with the variety Desiree. The average yield for all varieties was 47.14 t ha^{-1} and 39.66 t ha^{-1}. in 2013 and 2014 respectively. Variety Desiree had a minimal variation in yield, while the variety Aladin had the greatest variation. To achieve high yields of potatoes even distribution of rainfall is required and during the growing season it should be 350-400mm (Mitcherlich, 1971). Potatoes gives the highest yields with the annual precipitation of 700 to 800mm, while in very humid conditions (800-1000 mm) yield may be reduced by 4%. In 2013 during the vegetation period there was 359.5 mm of rain falls while in 2014 there was 610 mm, which significantly reduced the yield of potatoes in 2014.

Table 3. The total yield and the yield of marketable tubers

Varieties	Tuber yield (t ha^{-1})			Marketable tuber yield (t ha^{-1})		
	2013	2014	Average	2013	2014	Average
Agria	47.45	39.55	43.50	37.96	29.81	33.88
Aladin	52.90	40.25	46.57	44.43	31.28	37.86
Faluka	47.90	40.98	44.44	43.12	31.56	37.33
Kondor	50.72	43.12	46.92	41.08	31.91	36.49
Dezire	36.72	34.40	35.56	29.01	24.08	26.54
Average	47.14	39.66	43.39	39.12	29.73	34.42

	2013		2014		2013-2014	
	$LSD_{0.05}$	$LSD_{0.01}$	$LSD_{0.05}$	$LSD_{0.01}$	$LSD_{0.05}$	$LSD_{0.01}$
Tuber yield	5.765	7.111	3.278	4.602	2.823	3.806
Marketable tuber yield	4.505	6.321	2.475	3.474	2.405	3.242

The average yield of marketable tubers was 34.42 t ha^{-1}. In the two years examination the average yield the variety Aladin had the highest average yield of marketable tubers (37.86 t ha^{-1}), while the variety Dezire had the lowest one (26.54 t ha^{-1}). The varieties Agria, Aladin, Faluka and Kondor in comparison with the variety Desiree had statistically higher yield of marketable tubers. The varieties Aladin, Faluka and Kondor in comparison with the variety Agria had statistically higher yield of marketable tubers. In 2013, the variety Aladin had the highest yield of marketable tubers. In 2014, the variety Kondor had the highest yield. Variety Desiree had a minimal variation in yield, while the variety Aladin had the greatest variation.

For the further processing of potatoes, the size and shape of the tubers are important. Long and oval tubers larger than 50 mm are ideal for the preparation of French fries, while round tubers, between 40 and 60 mm, are ideal for the preparation of potato chips. Processing chips from tubers with a diameter greater than 60 mm would give large slices that would be damaged after packing. Size of the tuber is varietal characteristics, as well as the shape and irregular folds that lead to waste and losses during stripping. Irregular shape may be the result of secondary growth. Varieties with small folds are the most desirable for processing. Tubers with good circle pattern or oblong tubers can weight between 200 and 300 g, while larger tubers can weight even 500 g, and as such are acceptable for the preparation of French fries, but not for the chips. In addition to the shape and size of tubers the content of dry matter, sugars and color at the intersection of tubers are an important as well. If the dry matter content is too low, French fries or potato chips will be too soft or too moist and it is necessary to consume more energy when burning to evaporate water. If the dry matter content is too high, the finished product will be dry. Dry matter partly determines the texture and oiliness of finished products, which meet the needs of consumers. Potatoes with dry matter content of 20-24% are ideal for the production French fries, while those with dry matter content of 24% are ideal for the preparation of potato chips. As part of dry matter is lost during the peeling, cutting and blanching, it is better to have the higher initial dry matter content, as more content will remain after frying.

Variety Condor in both years had the highest starch content (18.97% and 18.22% in 2013 and 2014 respectively), while the variety Faluka had the lowest starch content (14.43% and 13.9% in 2013 and 2014 respectively). All studied varieties in comparison with the variety Faluka had statistically higher starch content in tubers. The variety Agria in comparison with the varieties Desiree and Aladin had statistically higher starch content, while Aladin in comparison with the variety Desiree had statistically significantly higher starch content.

Table 4 presents the results of dry matter content and starch in tubers. In both years the variety Agria had the highest percentage of dry matter (22.50%, and 21.27% in 2013 and

2014 respectively), while the variety Faluka had the lowest percentage of dry matter (17% and 16.37 % in 2013 and 2014 respectively).

Table 4. The content of dry matter and starch

Varieties	The content of dry matter (%)			The content of starch (%)		
	2013	2014	Average	2013	2014	Average
Agria	22.50	21.27	21.88	18.45	17.30	17.87
Aladin	19.10	17.87	18.48	16.33	15.25	15.78
Faluka	17.00	16.37	16.68	14.43	13.97	14.20
Kondor	21.77	20.70	21.22	18.97	18.22	18.60
Dezire	17.80	17.02	17.41	15.40	14.30	14.85
Average	19.63	18.65	19.13	16.72	15.81	16.26

	2013		2014		2013-2014	
	$LSD_{0.05}$	$LSD_{0.01}$	$LSD_{0.05}$	$LSD_{0.01}$	$LSD_{0.05}$	$LSD_{0.01}$
Dray matter	0.361	0.507	0.424	0.593	0.262	0.352
Starch	0.358	0.503	0.275	0.355	0.211	0.285

The variety Agria in comparison with other varieties had statistically higher dry matter content. The variety Kondor in comparison with varieties Aladin, Faluka and Dezire had statistically higher dry matter content. The variety Aladin in comparison with varieties Faluka and Dezire had statistically higher dry matter content, while the variety Dezire in comparison with variety Faluka had statistically higher dry matter content. The variety Agria can be used in processing to obtain French fries and potato chips. The dry matter content is an important indicator of the quality of potato tubers. Varieties of early maturation, as a rule, contain a smaller percentage of dry matter (***Quasemu***,1978; ***Đukić et. al***, 2000) which was confirmed by the results of these studies, since the early variety Faluka had the lowest dry matter content. ***Đokić et. al.*** (1988) found that the total dry matter content in tubers of some varieties of potatoes is in the range from 20 to 25%, of which starch accounts for 70-75% in average of the total dry matter or 14-26% of the fresh tubers.

The chemical composition of potato tubers depends on the genetic characteristics of the variety, soil and weather conditions, time of use, length of days, the number of plants per hectare and so on. Starch is a very important ingredient of tubers and significant in determining the value of potatoes for human consumption in the manufacturing industry (***Stoiljković***, 1987). It is known that the weather conditions during growth and development of plants have a significant effect on the starch content in the tubers. According to ***Huuniusa*** (1972) in complex weather conditions the effect of solar radiation, light intensity, air temperature, amount and distribution of rainfall have the most significant impact. Dry years bring low yields, but the tubers are rich in starch and moist, while cold and poorly sunny years have lower yields. **Suvajdžić and Šilić** (1984) considered that the temperature conditions were expressed as integral factors in the formation of yield and partially in quality of potatoes.

Conclusion

Based on the results obtained in the experiments the following can be concluded:

Varieties demonstrated a great variability in yield.

Variety Kondor had the highest average tuber yield (46.92 t ha^{-1}), while variety Aladin had the highest yield of marketable potato (37.86 t ha^{-1}).

Desiree variety had the lowest yield in general and in marketable potato tubers.

The highest dry matter content was found in variety Agria. The variety Agria is suitable for processing into French fries and potato chips.
Variety Kondor had the highest starch content.
By using quality seed and application of appropriate agricultural practices it is possible, in our agroecological conditions, to achieve high yields and high-quality tubers that can be used in the food industry.

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PERSPECTIVITY OAT GENOTYPES AND THEIR COMPONENTS OF PRODUCTIVITY AT THE PLOVDIV REGION (BULGARIA) AGRO-CLIMATIC CONDITIONS

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Abstract

During the 2010-2013 period, in the experimental field of Crop Science Department at the Agricultural University - Plovdiv a field experiment was conducted. Randomized complete block design was displayed with three replications and size of the experimental plot 10 m^2. Eight new Bulgarian wintering oat lines and two varieties (Dunav 1 and Resor 1), as a standards for yield and quality in Bulgaria, were researched. Yield structural elements formation in different oat genotypes, depending on the agro-meteorological features over the years was traced. The statistical processing of the experimental data was made by SPSS V.9.0 for Microsoft Windows. The highest number of tillers in all studied wintering oat genotypes formed line №1 (4,2) and line M-K (3,6). These two genotypes formed and highest number productive tillers. From all structural elements of panicle with highest number of spikeletts per panicle proven stands line 08/Z 2 (59,3), followed by 07 / Z 1 (27,3). Line 08 / Z2 formed and the highest number of grains per panicle - 98, but with less grain weight.

Keywords: *winter oat, genotypes, elements of the yield*

Introduction

The changes in the weather conditions significantly affect the development and thus determine the regions that are favourable for growing different varieties of crops, including grain crops (Kuzmova, 2009). Oats are a crop which prefers moist and cool weather, which is the reason why it is grown in these regions of the country (Dimitrova – Doneva, M., D. Tanchev, 1999). The changes in the climate necessitate creating new varieties that are more adaptable to the changing conditions. The high and the low temperatures of the air, the uneven distribution of the rainfall throughout the year, especially during the periods that are critical for the growth of the oats, significantly affect the development of the plants and thus the formation of the structural elements of the yield (Valchev, Dr., T. Savova, 1995; Savova, T., 1996). The shortening of the stages of development is caused by the high temperatures of the air and also by the lack of moisture in the soil (Palfner, 1991).The rise of the air temperature and the lack of sufficient rainfall during the spring-summer period shorten the period of ear formation, tasseling and ripening (Savova, T. 2002; Larson, 1988). The high temperatures and the lack of moisture from the end of the tillering period to the beginning of the stem elongation cause the growth of shorter panicles with fewer ears on them. The stem elongation is considered to be the most critical period of the development of the winter grain crops regarding the moisture in the soil. The conditions in which the tasseling takes place are also very important. The lack of moisture and the high temperatures during the formation of the grain lead to the growth of small and shrivelled grains. This adversely affects the yield and the quality of grain. The studying and the creation of high-yielding genotypes of winter oats adaptable to the certain agro-climatic conditions is the main purpose of this survey.

Materials and Methods

The field experiment was conducted within the period 2011-2013 in the experimental field of the Department for Plant Production at the Agricultural University – Plovdiv on the soil order Mollic Fluvisols based on FAO (Popova R., and Sevov, A., 2010).

8 winter lines of oats were included in the survey: № 1, 07/ Z1, 08/ Z2, M-K (selection of the Agricultural University-Plovdiv, Bulgaria), Radolishta, Kucevishte (selection from the town of Štip, the Republic of Macedonia), Kt 651, Kt 718 (selection of the Agricultural Institute – Karnobat, Bulgaria) and 2 winter cultivars – Dunav 1 and Resor 1 which are said to be yield and quality standards in Bulgaria.

The sowing was performed in mid October based on a block method in three repetitions, over an area of 10,5m^2 and a sowing rate of 500 k.s./ m^2 . The land had previously been planted with sunflower. The used fertilization rate was $N_6P_8K_8$.

The statistical processing of the experimental data was performed using SPSS V.9.0 for Microsoft Windows.

Results and Discussion

The weather conditions during the period of growth of the crop are the main factor determining the development of the plants and the formation of the structural elements of the yield.

Within the period of the survey, the vegetation year 2010-2011 was characterized as being favourable for the growth of oats.

The frequent and heavy rainfall in October delayed the sowing and as a result it was conducted in late November and the presence of low average temperatures of 10,8 °C (which is 1,8 °C below the norm) delayed the growth of the plants.

The period of panicle emergence, blossoming and grain formation was accompanied by heavy precipitations and the quantity of rainfall in May and June was above the norm while the temperatures were close to the long-term ones. The wax ripeness and the overall ripeness take place under temperatures that are 1,5 to 2,2°C above the norm (June-July) as well rainfall that is 5,5 mm above the norm (June). The combination of these conditions during the period of formation and ripening of the grains has a positive influence on the nutrition and some of the quality indicators of the grain. (Table 1 and Table 2).

Table 1. Average diurnal temperature in periods of ten days (°C), 2010 – 2011.

Months / Periods of ten days	X	XI	XII	I	II	III	IV	V	VI	VII
I	11,6	12,7	7	0,2	4,5	0,7	13	13,6	22,3	24
II	13,2	11,8	-1,8	4,6	2,9	9,2	10,2	17	22,5	26,7
III	7,8	9,5	2	-0,6	0,5	10,8	12,2	20,6	22,3	25,5
Average monthly temperature t° C	10,8	11,3	2,4	1,4	2,7	6,9	11,8	17,1	22,4	25,4
Average for the period 1965- 1995	12,6	7,4	2,2	-0,4	2,2	6	12,2	17,2	20,9	23,2

Table 2. Amount of the rainfall during the vegetation period (mm), 2010- 2011

Months Periods of ten days	X	XI	XII	I	II	III	IV	V	VI	VII
I	27,9	-	4,4	2,7	-	9,9	2	31,3	8,6	6,6
II	48,7	11	14,4	4,4	28,1	57,8	16,8	8,4	28,6	7,3
III	42,5	4,4	10,2	17,5	24	6,7	-	1,1	4,3	0,7
Monthly amounts	119,1	15,4	28,7	24,6	52,1	74,4	18,8	40,8	41,5	14,6
Average for the period 1965- 1995	47	35	36	40	48	44	39	32	36	42

The vegetation year 2011-2012 was not very favourable for the growth of the winter oats (Table 3 and Table 4).

The large quantity of the rainfall in October 2012 (70,4 l/m^2) again delayed the sowing of the winter genotypes of oats. This increased the likelihood of freezing of the plants. The positive temperatures in the winter months prevented the plants from freezing, owing to which the lines Radolishta and Kucevishte developed successfully. The spring months (March – April 2012) were characterized by favourable temperatures and rainfall, which determined the formation of panicles with more ears on them.

The period of grain formation and ripening (May-June) was characterized by temperatures close to and a little above the norm for the long-term period and also by heavy precipitations in May and quantity of the rainfall above the norm in June. This combination of weather conditions was favourable for the formation and the nutrition of the already existing grains on the spicelet, which is a prerequisite for a relatively high weight of the grains in the panicle.

Table 3. Average diurnal temperature in periods of ten days (°C), 2011- 2012

Months Periods of ten days	X	XI	XII	I	II	III	IV	V	VI	VII
I	15,3	7,3	3,4	-0,2	- 4,5	4,1	11,2	19,6	21,3	25,9
II	10,6	1,8	5,1	1,5	-3,1	8,1	14,3	16,5	24,6	27,5
III	8,7	2,4	-1,6	-3,0	3,5	13,2	17,7	16,6	24,9	27,5
Average monthly temperature t° C	11,6	3,8	2,3	-0,6	-1,4	8,4	14,4	17,6	23,6	27,0
Average for the period 1965- 1995	12,6	7,4	2,2	-0,4	2,2	6	12,2	17,2	20,9	23,2

Table 4. Amount of the rainfall during the vegetation period (mm), 2011- 2012

Months Periods of ten days	X	XI	XII	I	II	III	IV	V	VI	VII
I	22,8	0,0	0,4	65,6	47,2	2,0	13,5	37,3	40,1	0,0
II	38,3	0,8	12,6	0,0	2,6	2,7	8,2	73,7	0,0	2,0
III	9,3	0,1	25,8	54,6	7,0	0,2	0,5	49,8	2,3	0,4
Monthly amounts	70,4	0,9	38,8	120,2	56,8	4,9	22,2	160,8	42,4	2,4
Average for the period 1965- 1995	47	35	36	40	48	44	39	32	36	42

The third vegetation year 2012-2013 was characterized by high temperatures and rainfall unevenly distributed during the months (Tables 5 and 6). The high temperatures in October and the rainfall above the norm allowed the fast and simultaneous germination of the crops. This guaranteed the normal duration of the period of development, the occurrence of the third-leaf phenophase and the initiation of the tillering stage before the drop of the temperatures. During the winter months, positive temperatures above the norm were registered, which contributed to the successful wintering of the plants. However, the small quantity of the rainfall in that period reduced the moisture of the soil which is necessary in the spring. In February and March, when the spring vegetation starts, the rainfall is below the norm, which shortens the duration of the tillering stage. This has a negative effect on the formation of the overall and the productive tillering of the plants.

Table 5. Average diurnal temperature in periods of ten days (°C), 2012- 2013

Months Periods of ten days	X	XI	XII	I	II	III	IV	V	VI	VII
I	17,4	11,4	3,4	1,4	4,5	7,0	11,1	19,4	18,6	21,8
II	16,2	7,9	-0,2	2,0	3,8	8,0	13,2	20,7	23,3	23,2
III	12,7	6,8	-0,3	2,0	5,5	7,2	17,8	20,0	23,1	25,1
Average monthly temperature t° C	15,6	8,7	0,97	1,8	4,6	7,4	14,03	20,03	21,67	23,37
Average for the period 1965- 1995	12,6	7,4	2,2	-0,4	2,2	6	12,2	17,2	20,9	23,2

Table 6. Amount of the rainfall during the vegetation period (mm), 2012- 2013

Months Periods of ten days	X	XI	XII	I	II	III	IV	V	VI	VII
I	0,3	0,3	40,6	2,8	8,5	0,2	74,9	1,3	33,7	53,4
II	2,0	1,7	58,0	4,4	19,1	3,9	9,7	0,1	28,1	1,6
III	37,1	8,7	0,1	30,1	17,5	29,8	0,0	2,0	47,7	8,3
Monthly amounts	39,4	10,7	98,7	37,3	45,1	33,9	84,6	3,4	109,5	63,3
Average for the period 1965- 1995	47	35	36	40	48	44	39	32	36	42

In May, when the plants are in their tasseling and milky ripeness stages, minimum quantity of the rainfall of 3,4mm/m^2 was registered, considering the fact that the norm for the long-term period is 32mm/m^2 . These conditions and temperatures 2,8° C above the norm provide prerequisites for the abnormal pollination of the blossoms of the panicle and the growth of a smaller number of grains. This disrupts the normal formation of the grains.

The heavy rainfall in June - 109,5mm/m^2 contributed only to the nutrition of the grains already formed in the panicle. Under these conditions, we have well-nurtured but fewer grains on the panicle.

The tillering of the studied genotypes varied widely over the years depending on the agro-climatic conditions (Table 7). For the Macedonian genotypes Radolishta and Kucevishte, this indicator has not been registered during two of the years of the experiment because of the large percentage of freezing of the plants.

The largest number of tillers was registered for line No. 1 – 4,2 tillers, followed by line M-K – 3,6 tillers. These two lines have the largest number of productive tillers between 2,2 and 2,4. The lowest overall tillering was registered for line 08/Z2 – 2,1 tillers. Of all the studied

genotypes, the least productive tillers on average for the entire period of survey were registered for the cultivars Resor 1.

Table 7. Overal and productive tillering (number of tillers/ plant), 2011 – 2013.

Genotypes	Overall tillering				Productive tillering			
	2011	2012	2013	Average	2011	2012	2013	Average
№ 1	4,2	4,0	4,4	4,2	2,4	3,7	1,1	2,4
07/ Z 1	2,8	2,6	3,0	2,8	2,0	1,6	1,2	1,6
Dunav 1 - st	3,6	3,1	3,5	3,4	1,9	2,7	1,1	1,9
08/ Z 2	2,3	1,5	2,5	2,1	1,7	2,0	1,7	1,8
M-K	3,8	3,7	3,3	3,6	2,2	2,1	2,3	2,2
Kt 651	2,4	3,6	4,2	3,4	1,4	2,5	2,4	2,1
Resor 1 - st	3,4	1,8	2,0	2,4	2,1	1,1	1,0	1,4
Kt 718	3,6	3,0	3,6	3,4	2,0	1,8	2,2	2,0
Radolista	-	2,1	-	2,1	-	1,9	-	1,9
Cuceviste	-	2,2	-	2,2	-	2,0	-	2,0

Regarding the length of the panicle, table 8 shows that there are great differences between the winter genotypes of oats included in the experiment. The shortest panicle is that of line 07/Z1, reaching 16,8 cm and the longest one is that of line 08/Z2 – 20,8 cm. These two lines are distinguished from all the rest for having the largest number of spicelets, the largest number of grains on the panicle and, respectively, the highest weight of the grains on the panicle. The panicle with the largest number of ears (59,3) and the largest number of grains (98) is that of line 08/Z2. Regarding these indicators, it surpasses all the other genotypes included in the survey but is inferior in terms of size of the grain (with the exception of lines Radolishta and Kucevishte) and hectoliter weight (Zorovski, P et al, 2014).

Table 8. Structural elements of the panicle at wintering oat genotypes, average for the period of study 2011-2013.

Genotypes	Panicle length (cm)	Spikelets per panicle (number)	Grains per panicle (number)	Grain weight per panicle (g)
№ 1	20,0 a	24,2 c	47,2 b	1,06 a
07/ Z 1	16,8 b	27,3 c	54,6 b	1,60 a
Dunav 1 - st	20,4 a	24,5 c	44,2 b	1,23 a
08/ Z 2	20,8 a	59,3 a	98,0 a	2,00 a
M-K	19,7 a	24,0 c	40,4 bc	1,13 a
Kt 651	18,9 a	21,9 c	42,2 b	1,01 a
Resor 1 - st	19,7 a	31,6 b	56,6 b	1,21 a
Kt 718	16,3 b	26,0 b	40,1 bc	1,33 a
Radolista	17,9 b	19,2 d	40,0 bc	0,98 b
Kuceviste	18,2 b	21,0 c	43,1 b	1,21 a

Conclusions

The conditions throughout the year largely affect the degree of tillering of the studied winter genotypes of oats. The largest overall and productive tillering was registered for line No. 1, followed by line M-K. Of all the studied genotypes, the largest number of spicelets on a panicle was registered for line 08/Z2 (59,3), followed by line 07/Z1 (27,3). These two genotypes also have the largest number of grains on the panicle and the largest weight of the grains on the panicle. They prove to be the most suitable to be grown in the respective agro-climatic region.

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DESIGN DEVELOPMENT OF MULTI-NUTS CUTTING TOOLS

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Abstract

The Present research work introduces a multi-nut post harvesting mechanism in single platform for shelling, cracking, cutting, de-hulling like operations by just exchanging a tool and fixture set. The different types of nuts such as Marking-nuts, Wal-nuts, Cashew-nuts, Areca-nuts, Almond, Amla, Pistachio, Mango etc. by using a same vertical cut plane post-harvesting mechanism. The Computer Aided Engineering Analysis (CAE) by Finite Element Method (FEM) to perform a simulation for design optimization MNHM (Multi nut harvesting Machine) is done by considering an actual processing condition parameters to perform a simulation cycle for the structural analysis. The post processor report resulted that sum of the reaction forces 38482N (along X-axis), -3641N (anticlockwise along Y–axis) and 20757.5N (Z-axis). The average Product material efficiency of multi nut dehuller for *marking nut, Almond, Walnut and Areca nut*: 87.22, 85.29, 72.23 and 97.00%. Average mechanical damage: 12.78, 14.71, 27.77, and 0.0%, whereas throughput capacity: 0.60, 0.60, 2.48 and 3.5kg/hr (areca of 0-15 mm) respectively. The average calculated parameters for 100 pieces in MNPH-processing unit mechanism observed Product material efficiency: 87.37%, operational time: 85.29%, Shelling efficiency: 97.64%, Mechanical damage: 11.37%, Throughput capacity: 1.17 (Kg/hr), the punches, cutters and MNPH processing unit tools are easier to exchange. The Cost of modified machine is Rs.3100.00/- ($50).

Keyword: *Multi-nuts, ergonomic analysis, CAE analysis*

Introduction

The Present research work introduces a flexible multi-nuts shelling,cracking,cutting , dehulling like operations by just exchanging a tool and fixture set .The different types of nuts such as Marking-nuts, Wal-nuts, Cashew-nuts, Areca-nuts, Almond, Amla, Pistachio etc. by using a same vertical cut plane post-harvesting mechanism. The use of CAD/CAM/CAE-Software technology results in identifying scope in designing and redesigning the machine tool components by structural analysis in software environment.

Objectives of proposed research work:

To Study the existing Shelling, Cutting, Cracking and Dehulling Mechanisms.

Preparation of a new conceptual design of tools and mechanism in Software
-environment for simulation cycle and confirmation of design specification.

Fabrication and performance evaluation of the MND-machine tools.

Ergonomic performance evaluation of MND-Machine.

Materials and methods

The study of the operational functionality of the multi-nut post harvesting mechanism, during de-hulling by stone hammering most of the seed kernels becomes blackish so their market value decreases. The breaking of marking nut is very tedious, laborious and time consuming in traditional method. There were some dehuller like curved blade type dehuller, notch type dehuller, shell and blade type dehuller, hold and press type dehuller designed by other researchers. Following points were considered in the design of MNPH-Processing Unit Mechanism.

- Separation of kernel without or with less damage in cracking.
- More than one kind of nuts for their cutting, slicing, cracking, squeezing, shelling and DE hulling like operations separate processing tools are designed to operate in single mechanism.
- Prevention of splashing during cracking on the facing body parts of the workers.
- Minimization of efforts of the workers in cracking.

The physical dimensions of nuts derived for designing the cutting tool geometry specification to be fixed in vertical cut plane mechanism as mentioned below in Table No.1.

Table No.1. Physical dimensions for cutting tool geometry design specifications

Nuts	Physical Dimensions	Operation Type / Force
Marking Nut	H=18.94 to 26.45; W=15.74 to 18.70; t =1.86 to 2.78; m=2.36 to 2.57.	cracking , shelling
Walnut	H= 34.25 to 40.80; W=27.58 to 32.50, t=2.80 to 3.41; m= 9.13 to 13.27.	cracking , shelling
Almond Nut	H=34.25 to 40.80; W=27.58 to 32.15; t=2.82 to 3.41; m=9.13 to 13.27.	cracking , shelling
Areca Nut	H=20.98 to 22.87; W=29.49 to 30.23; m=14.78 to 16.45.	slicing
Aonla	D =40.7; d (seed) =15; m=35.	Hollow cutting
Mango	H=4 -10; W= 3 –6; h= 2– 6; w = 2– 4.	cutting ,slicing

Where H-height in mm, W-width in mm; m-mass in gm.; t-thickness in mm, d-diameter of seed, h-height of seed.

Design consideration:

Following points were considered in the design of MNPH-Processing Unit Mechanism.

- Separation of kernel without or with less damage in cracking.
- More than one kind of nuts for their cutting, slicing, cracking, squeezing, shelling and DE hulling like operations separate processing tools are designed to operate in single mechanism.
- Prevention of splashing during cracking on the facing body parts of the workers.
- Minimization of efforts of the workers in cracking.

Ergonomic Evaluation:

Statistical analysis of anthropometrics data

The design of agricultural implement or tractor requires anthropometric data of user population for enhancing operator’s comfort, safety and efficiency. The selected anthropometric data of Indian agricultural workers are given in Table.2. (IS 12239/1:1996)

Table.2: Normal range anthropometric data of Indian agriculture workers

Dimensions		Male	Female
Height (mm)		1660-1820	1490-1745
Weight (kg)		55-85	45-60
Hand length (mm)	Full	740-840	655-750
	Elbow	40-50	35-45
Hand grip dia (mm)		55-65	50-60
Shoulder height (mm)		1144-1569	1083-1420
Eye height (mm)		1336-1685	1230-1560

Statistical analysis of anthropometric data of 10 selected healthy operators, which is presented in table No. 3.

Table No.3: Statistical analysis of anthropometrics data

Sr. No.	Height (mm)	Weight (Kg)	Hand length (mm)		Hand grip dia (mm)	Shoulder height(mm)	Eye height (mm)
			F_L	E			
1 (M)	1661	57	683	42	58	1381	1571
2 (M)	1672	67	740	44	63	1362	1662
3 (M)	1685	69	761	46	64	1395	1595
4 (M)	1640	80	732	44	62	1340	1540
5 (M)	1641	75	738	45	60	1371	1551
6 (M)	1654	62	750	46	57	1364	1564
7 (M)	1680	61	747	47	59	1380	1580
8 (F)	1676	50	740	42	53	1396	1576
9 (M)	1732	70	770	48	65	1412	1632
10 (F)	1665	48	720	43	52	1361	1561
Total	16706	639	7381	447	593	13762	15832
Average	1670.6	63.9	738.1	44.7	59.3	1376.2	1583.2
S.D	26.475	10.332	23.969	2.057	4.423	21.038	37.573
5^{th} per.	A 1641	50	720	42	53	1361	1551
	B 155	42.0	735	45	55	1390	1448
50^{th} per.	A 1665	62	740	44	59	1371	1571
	B 1645	52.5	754	50	60	1470	1549
95^{th} per.	A 1680	70	750	46	63	1395	1595
	B 1745	63.0	798	60	65	1520	1647

F_L-Full hand length up to fingertip and E-Hand length from elbow joint, M- Male and F-female, A- As per 10 samples and B- as per CIAE Bhopal

Table.4.Average range of blood pressure and heart rate

Age group	Diastolic (mmHg)	Systolic(mmHg)	Pulse rate(bpm)
Infants	65	95	80 - 160
Children (6-9yrs)	65	100	75-115
Adults	60 – 80	90 – 120	80-120

Table.5.Blood pressure and pulse rate measurement of operators

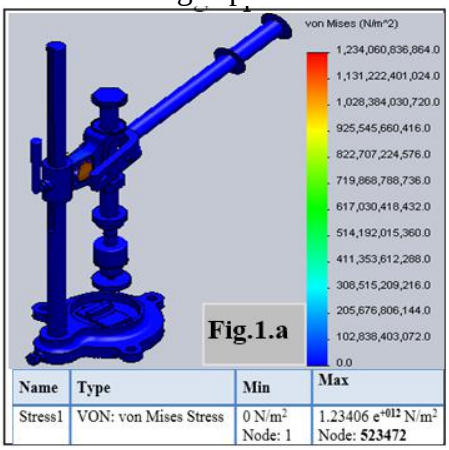
Operator No.	Blood pressure and pulse rate at rest position			MNPH Mechanism (Average values with all cutter			Vibrations during operations m/s^2
O	**A**	**B**	**C**	**A**	**B**	**C**	**Ac**
1 (M)	64	115	90	78	120	95	4.8
2 (M)	80	119	89	82	124	98	4.3
3 (M)	84	120	108	84	127	116	4.6
4 (M)	78	110	96	85	125	105	4.7
5 (M)	75	108	85	80	118	98	4.5
6 (M)	79	112	94	85	120	106	4.9
7 (M)	72	104	106	85	129	119	5.2
8 (F)	68	115	99	78	121	114	5.4
9 (M)	76	118	113	85	129	120	6.0
10 (F)	69	112	102	78	123	118	5.3

O-operator No.; M- Male operators; F-female operators; A- Diastolic blood pressure (mm/Hg);

B- Systolic blood pressure (mm/Hg); C- Pulse rate (bpm), Ac- Vibration counts in m/s^2.

Table 6.shows the two types of blood pressure of male and female operators of MNPH Machine and vibrations measured during operations for each operator. It was observed that blood pressure and pulse rate increases in moderate limit and the vibrations are also within

safe limit. The cutting tools and respective fixtures after CAE-simulation cycle see in fig1.a confirmed design specifications. The CAD-drawings of all cutting tools mentioned in fig.1.b



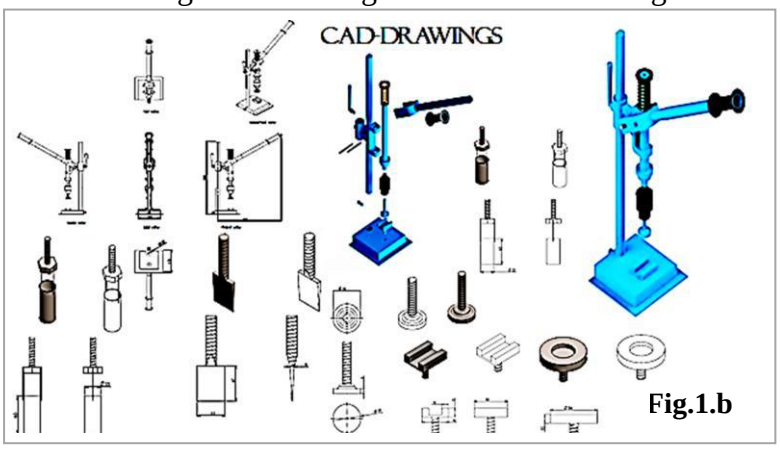


Fig1.**a**.CAE-Simulation of MNPH machine; **b**. CAD-drawings of MNPH cutting tools and fixtures

Results and discussion

The performance evaluation of marking nut, walnut, Almond, Areca nut as shown in fig.2. and according to ergonomic evaluation the performance by each operator for different nuts and tools as from table 5. is as shown in fig.3.for blood pressure ,pulse rate and vibration effect after operation. The overall performance for all selected four nuts and two fruits such as aonla and mango on MNPH machine are graphically represented as in fig.4 and 5 .

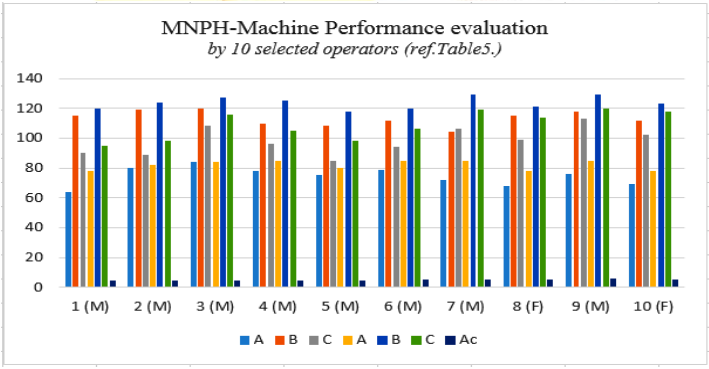
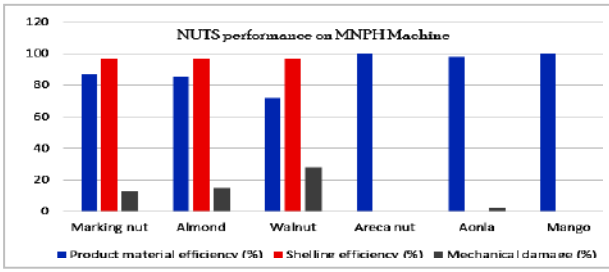


Fig.2. Performance test of MNPH machine

Fig.3. Performance by each operator for different nuts



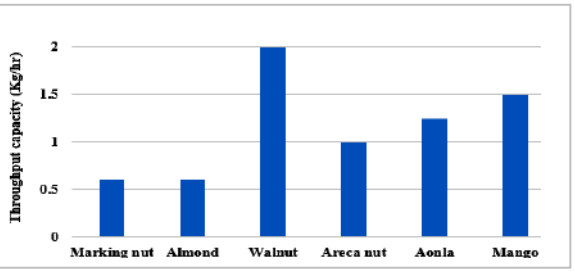


Fig.4. MNPH machine performance Fig.5. Throughput capacity (Kg/hr) of MNPH

Conclusion

The Computer Aided Engineering Analysis (CAE) by Finite Element Method to perform a simulation for optimization MNPH Machine is done by considering an actual field condition parameters to do the structural analysis and also ergonomic evaluation according to ISO and ILO standards for operator machine interface. It is experienced to be comfortable with less fatigue in operator's energy cycle.

The sum of the forces in the structural simulation along X-axis:38482N, Y–axis:-3641N, Z-axis: 20757.5N was observed. The structural analysis resulted in safe limit for all parts.

It was observed that blood pressure and pulse rate increases in moderate limit and the vibrations are also within safe limit. It is observed that throughput capacity (Kg/hr) of marking nut, walnut, areca nut, aonla and mango was ranging from 1-3kg/hr.

The cutting tools with accessories in MNPH machine for processing operations are easier to exchange.

The Cost of modified machine is Approx.$50.00(Approx. INR 3100)

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USING PRINCIPAL COORDINATES ANALYSIS FOR INTERPRETING GENOTYPE × ENVIRONMENT INTERACTION IN PLANT BREEDING PROGRAMS

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Abstract

One of the major breeding objectives for many crops is improved yield performance and after identifying the high yielding genotypes it is essential to evaluate them for yield stability over diverse environments. The success of any breeding program depends on several factors, including understanding and selection of suitable breeding and test locations. Conventional statistical models of stability analysis provide little or no insight into the pattern of the GE interaction while the principal coordinates analysis can account more effectively for the underlying GE interaction patterns. According to grand means of test environments and total mean yield, environments are classified to two main groups as H (high mean yield) and L (low mean yield). The identified most stable and high mean yield genotypes (G5) are based on the minimum spanning tree plots and centroid distances which could be recommended for unfavorable or poor conditions. Also, genotypes (G11) which were located more times in the vertex positions of high cycles, according to the principal coordinates analysis, are the most stable ones with high mean yielding properties. The principal coordinates analysis can provide useful and interesting ways of investigating GE interaction of genotypes. Finally, the results of principal coordinates analysis in general confirmed the breeding value of crop genotypes, obtained on the basis of the yield stability evaluation. Such an outcome could be regularly employed in the future to delineate predictive, more rigorous recommendation strategies as well as to help define stability concepts for recommendations for crops in other areas of the world.

Keywords: adaptation, GE interaction, stability, yield performance

Introduction

Plant breeders perform multi-environment trials for evaluation of new improved genotypes across test environments, before a specific genotype is released as a commercial cultivar to be produced by growers. In such experiments, genotype × environment (GE) interaction is a common phenomenon (Kang, 1998, Annicchiarico 2002; Yan et al. 2007) which is refer to the differential ranking of genotype among environments and may complicate the process of selection and recommendation of genotypes to target environments (Ebdon and Gauch, 2002; Gauch, 2006). It may also reduce the efficiency of selection in different breeding programs because in the presence of GE interaction, the measured trait is less predictable and cannot be interpreted using main effects and need more analysis (Gauch et al. 2008). Also, GE interaction is one of the most important reasons for the failure or efficiency decrease of breeding efforts to serve small resource-poor farmers in the arid and semi-arid areas (Ceccarelli et al. 2006). Plant breeders perform multi-environment trials to select favorable genotypes based on the both mean yield and stability performance, and to determine whether the test environment is homogeneous or should be divided into various mega-environments (Yan and Kang, 2002; Gauch, 2006).

The adaptation strategies maybe focus on responses of a set of genotypes across different test environments to obtain some good predictions because the plant materials may be produced

from the genetic bases of which the tested genotypes are assumed to be a representative sample (Annicchiarico, 2002). The adaptation strategies maybe focus on cultivar recommendation which is involves several statistical methods including parametric univariate, nonparametric univariate, and multivariate procedures (Flores et al. 1998). Among these methods, the most widely used is the joint regression model (Finlay and Wilkinson, 1963) which is uses only one statistic, the regression coefficient, to describe the pattern of response of a genotype across environments and, most of the information is wasted in accounting for deviation. Principal components (PC) analysis is a common generalization of above model that overcomes this difficulty by using the scores of PC axes as an extra statistic to explain the response pattern of a genotype (Eisemann et al., 1990).

Principal coordinates analysis is a generalization of famous PC analysis and involves with measurement of similarity between different variables such as genotypes or environments (Gower, 1966; Westcott, 1987). This method assumes that the original variables define a Euclidean space and so the similarity between them is modeled by Euclidean distance and its main target is to transform the data from one series of coordinate axes to the other series (Medina et al., 1999). Like PC analysis, this method preserves most of the original configuration of the dataset in the first axes and so, some original information is inevitably lost. The principal coordinates analysis can effectively reduce the pattern of a two-way dataset of multi-environmental trails, dimensions in a subspace of fewer dimensions (Ibanmez et al., 2001). Also the mentioned two-way structure can be conceptualized as environment points in genotype dimensions.

Ordination approaches such as the principal coordinates analysis may have some limitations. In dimension reduction of dataset, distortions may occur. In other word, if the percentage of variance explanted by the first axes is small, individuals that are really far part may be indicated by points that are close together (Gower, 1971). Also, a lack of association between variables prevents few dimensions from explanting for most of the observed variation. In contrast to ANOVA (additive model), multivariate methods such as the principal coordinates analysis assumes a multiplicative model without any explanation of the main effects (Zobel et al., 1988). In some cases the first axes of multivariate methods do not have any clear association to environmental factors (Gauch et al., 2008). Finally, the nonlinear relationships prevent from effective explanation of the real relationships between genotypes through multivariate methods (Gower, 1971). The aim of the present study was to quantify and interpret the GE interaction on performance stability using principal coordinates analysis which should help to interpreting genotypes adaptability for grain yield, which is a complex trait particularly susceptible to GE interaction.

Principal coordinates analysis

Analysis of variance was performed for individual environments to plot residuals and identify outliers. The Anderson-Darling normality test and the Levene variances homogeneity test were assessed. Each of the four location and 18 genotypes were regarded as fixed variables while three years were regarded as random variables. Combined analysis of variance was conducted using by SAS 9.1 program (SAS, 2004). The principal coordinate analysis (Westcott, 1987) was used for stability analysis. A measure of similarity between two genotypes, *m* and *n*, in a given test environment is:

$$S_{i(m,n)} = [H_i - (m_i + n_n)/2]/(H_i - L_i)$$

where *Hi* is the highest mean yield of a genotype in test environment *i* ; *Li* is the lowest mean yield of a genotype in test environment *i* ; m_i is the mean yield of genotype *m* in test environment *i* and n_i is the mean yield of genotype *n* in test environment *i*. Similarity index between two genotypes (*m* and *n*) was defined as the average of $S_{i(m,n)}$ across test environments when more than one test environment was used. The analysis was based on the sequential accumulation of the test environments according to their rank order, the

environments being ranked in ascending order according to their overall means. Each analysis produced a two-dimensional plot based on the first two principal coordinates scores. Also, the minimum spanning tree plots were drawn and the most stable genotypes with high mean yield performance were those that across sequential cycles were observed most distant from the centre of the plot. All calculations and plots of the principal coordinate analysis were performed by GENSTAT 12.1 software (VSN International, 2009).

Interpretation

According to these grand means and total mean yield, test environments are classified to two main groups as H (high mean yield) and L (low mean yield). There are seven H test environments and five test environments which analyzed in the sequential cycles. Yield is first analyzed for the lowest test environment (cycle L1); the second cycle (L2) involves analyzing the two lowest environments, and so on. A typical plot for the cycles is shown in Figure 1 where the scatter point diagram indicates the results of analysis for the first low cycle (L1). Plot of first two principal coordinates analysis axes in cycle L1 (Figure 1) indicated genotypes G7 and G10 were different from the other genotypes.

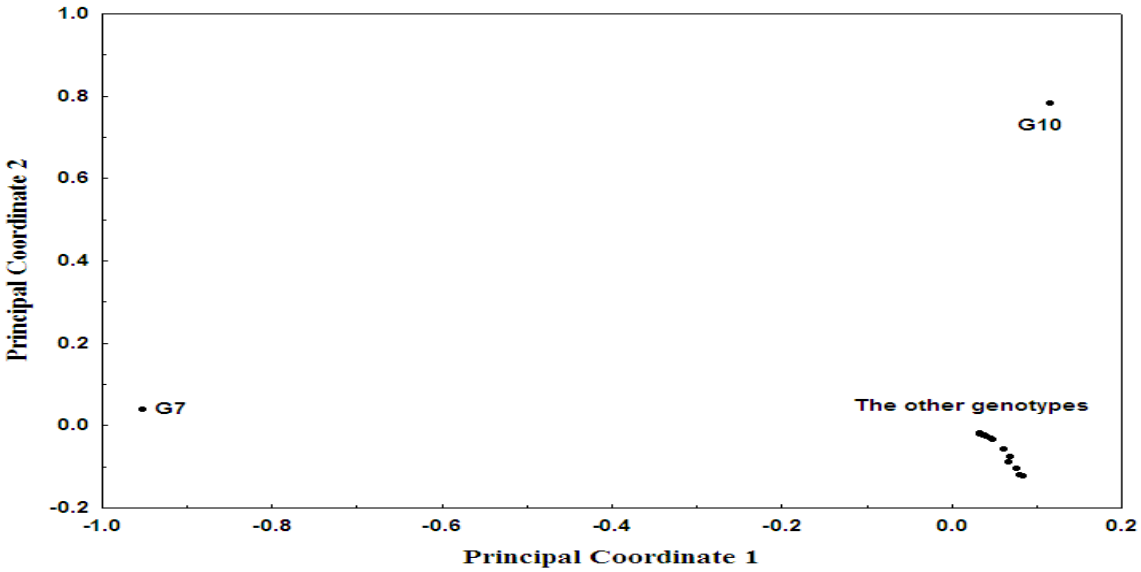

Figure 1. Plot of the first two principal axes from a principal coordinate analysis in twelve environments, in cycle L1.

This plot uses only first two principal coordinates analysis axes and so ignoring some information of the other principal coordinates axes and a minimum spanning tree plot could be useful. The high-yielding genotypes are those which are furthest from the centre, and so genotypes G1, G2 and G11 were detected as the high yielding genotypes in L5 cycle (Figure 2).

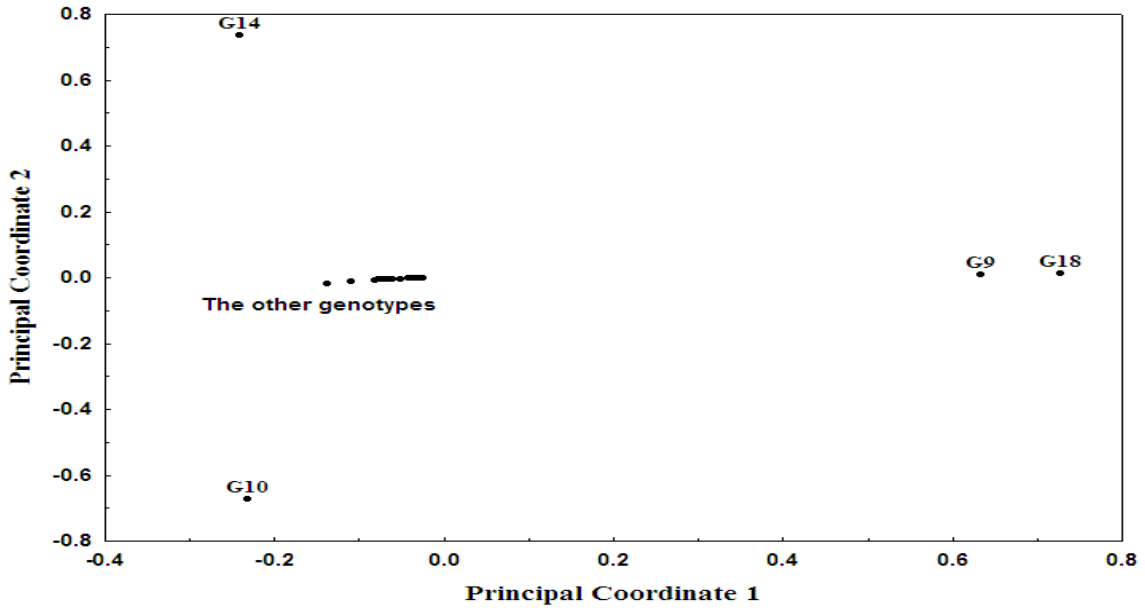

Figure 2. Plot of the first two principal axes from a principal coordinate analysis in twelve environments, in cycle H1.

The differences in the lengths of the branches are grotesque relative to the differences between studied genotypes, because the minimum spanning tree is represented in two dimensions ignoring information in the next principal coordinates axis. Regarding this limitation, Flores et al. (1996) proposed using a parameter as centroid distances which is benefits from all principal coordinates dimensions. Rather than including all five scatter diagrams of L cycles, the stability patterns of the genotypes are described in the text and only centroid distances, corresponding to all L cycles are presented. According to centroid distances values, genotypes G2, G3 and G7 were the favorable genotypes in cycles L1 and L2 while genotypes G1, G2, G11 and G12 were the favorable genotypes in cycles L3, L4 and L5. According to obtained results for all low cycles, genotypes G1, G11 and G14 were the favorable stable ones. Among these genotypes only G2 and G11 were located three times in the vertex positions of five low cycles and so these genotypes (G2 and G11) were the most stable ones with high mean yielding properties. The most stable genotypes are the ones that are consistent over cycles (Westcott, 1987; Flores et al., 1996). The identified most stable and high mean yield genotypes had acceptable mean yield; G2 and G11, and therefore could be recommended for unfavorable or poor conditions.

Seven test environments were as H (high mean yield) which analyzed in the sequential cycles. Yield is first analyzed for the highest test environment (cycle H1); the second cycle (H2) involves analyzing the two highest test environments, and so on. According to scatter point diagram of the first high cycle (H1), genotypes G9, G10, G14 and G18 were different from the other genotypes (Figure 3).

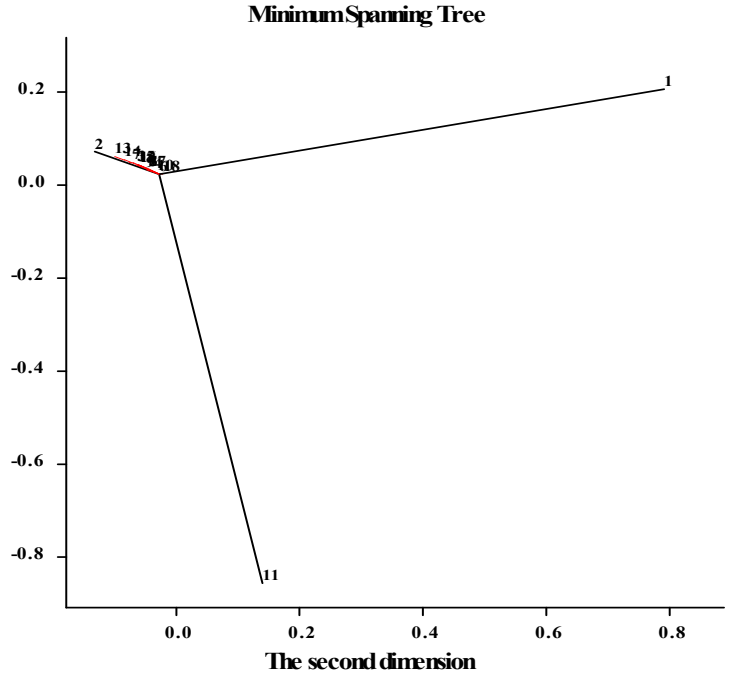

Figure 3. Plot of the first two principal axes from a principal coordinate analysis in twelve environments, in cycle L5. Part of the minimum spanning tree is superimposed on the plot.

Similar to L cycles, minimum spanning tree plot was used and showed that genotypes G5, G9 and G14 were high-yielding genotypes because they were furthest from the centre in H5 cycle (Figure 4).

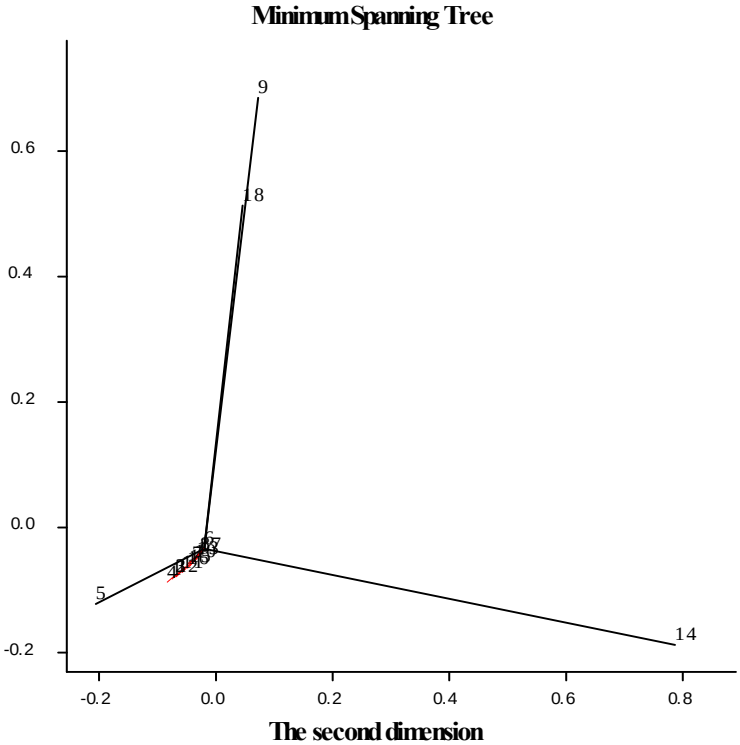

Figure 4. Plot of the first two principal axes from a principal coordinate analysis in twelve environments, in cycle H5. Part of the minimum spanning tree is superimposed on the plot.

Rather than including all seven scatter diagrams of H cycles, the stability patterns of the genotypes are described using centroid distances. According to centroid distances values, genotypes G2, G10 and G14 were the favorable genotypes in cycle H1; genotypes G9, G10

and G14 were the favorable genotypes in cycle H2; genotypes G5, G9 and G18 were the favorable genotypes in cycle H3; genotypes G9, G14 and G18 were the favorable genotypes in cycle H4; genotypes G5, G9 and G14 were the favorable genotypes in cycles H5 and H6; genotypes G1, G2, G5 and G14 were the favorable genotypes in cycle H7. Among these genotypes only G5, G9 and G14 were located four, five and six times in the vertex positions of seven high cycles, respectively and so these genotypes were the most stable and high yielding.

Discussion

Breeder's awareness of the importance of GE interaction has led genotypes to be ordinarily assessed in multi-environment trials for recommendation to farmers or for the final stages of elite breeding material selection. Many complex traits like grain yield are influenced by both genetic and environmental factors but GE interaction has considerable affect on this trait (Yan et al. 2011). The GE interaction makes it difficult to select favorable genotypes that produce high yields and that are more stable in plant breeding programs and reduces the selection progress. The investigation of the GE interaction permits the classification of genotypes by their behavior in two different environmental conditions. There are many statistical methods available to analyze the GE interaction including combined ANOVA, univariate stability analysis, nonparametric methods and multivariate procedures. Combined ANOVA is often used to detect the existence of GE interaction in multi-environmental trials but its main limitation is the assumption of homogeneity of variance among environments needed to determine genotype differences (Annicchiarico, 2009). Among univariate stability analysis, linear regression model was used extensively but it has several limitations from both the biological and statistical points of view.

The principal coordinate analysis seems necessary for an adequate description of the GE interaction, because conventional methods confound GE interaction and main effects and are unable to explain non-linear genotypic response to the environments. This method appears to be able to extract a large portion of the GE interaction and is thus more efficient in analyzing GE interaction pattern in different crops, as demonstrated by Flores et al. (1996), Medina et al. (1999) and Ibanmez et al. (2001). Also the detected most favorable genotypes in both cycles or condition (favorable versus unfavorable) are relatively the most high yielding genotypes and so it seems that this strategy can select the most stable genotypes based on the dynamic stability concept. However in the semi-arid regions and rain fed condition, where fluctuations in growing conditions are unpredictable, additional investigations are needed to obtain an integration of GE interaction analysis with environmental factors.

Multivariate statistical analysis has three main targets; elimination noise in the data set, summarizing the information and reveal a pattern in the data (Gauch, 2006). Methods based on PC analysis, such as AMMI and SREG, are linear-bilinear models with an additive component and a multiplicative component (Gauch et al., 2008). The SREG model is recommended when the environments are the main source of variation in relation to the contributions of the genotypes and the GE interaction with respect to the total variability. Our principal coordinate analysis results are useful for comparing the merits of different crop genotypes, and show which ones are capable of stability across different environmental conditions. Flores et al. (1996) found that both AMMI and principal coordinate analysis procedures obtained equally satisfactory results while Medina et al. (1999) reported that principal coordinate analysis might be more straightforward than AMMI model when there are values that are conspicuously separated from the majority of other values. According to this investigation, for the crop genotypes, the principal coordinate analysis seems necessary for an adequate description of the GE interaction. The present dataset and other similar studies (Flores et al., 1998; Ibanmez et al., 2001) encountered problems, because most conventional

stability models confound GE interaction and main effects and are unable to explain non-linear genotypic response to the environments.

Also, the identified most favorable genotypes in both cycles (favorable versus unfavorable conditions) were the highest yielding genotypes. However in the semi-arid regions and rain fed condition, where fluctuations in growing conditions are unpredictable, additional investigations are needed to obtain an integration of GE interaction analysis with environmental factors. The yield stability refers to a genotype's ability to perform relatively consist across a range of environmental conditions. The stability approaches relate to either of two contrasting concepts of stability as static and dynamic. From dynamic stability concept implies for a stable genotype a response in each test environment that is parallel to the mean response of the tested genotypes. It seems that the results of principal coordinate analysis are mostly associated with the dynamic concept of stability. The development and use of principal coordinate analysis method can enable incorporation of stability in the selection process. There are several methods of simultaneous selection for mean yield and stability performance and relationships among them.

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EXAMINATION OF SOME DUTCH WHITE FLESH POTATO VARIETIES IN MOUNTAINOUS REGION OF MONTENEGRO

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Abstract

The paper presents results of productivity of five Dutch potato white flesh varieties in the mountainous regions of Montenegro (Nikšić, 800 meters of altitude). The experiments were conducted during 2013 and 2014, and following varieties were tested: Arrow, Messi, Volare, Dido and Kennebec. Variety Kennebec was used as a standard. The highest tuber yield in two-year average had variety Messi (37.1 t.ha^{-1}), while the lowest yield was measured in the crop of Kennebec variety (27.3 t.ha-1). Variety Messi had significantly higher yields compared to all other investigated varieties. Variety Messi yielded averagely biggest tubers (109.9 g), and established differences in size of tubers compared with all other varieties are indicated as statistically significant. The largest numbers of tubers in the experiment was determined in varieties Dido (10). Increased the number of tubers in Dido variety in comparison with all others was statistically significant.Higher potato yields were obtained in 2014 (35.1 t.ha^{-1}) as the result of the higher total amount of rainfall during the potato vegetation period and better monthly distribution as well.

Key words: white flesh potato varieties, number of tubers, average tuber weight, yield

Introduction

Potato (Solanum tuberosum L.) is the most important non-cereal crop in the world (http://faostat.fao.org). Potato is one of the most important agricultural cultures in Montenegro as well and it is particularly important for the people in the mountain area of Montenegro. In the structure of production on arable land potato is planted on just over 20% (http://www.monstat.org).

Several thousand of potato varieties exist in the world today, among which varieties with yellow skin and flesh colour dominate. Despite this, the most cultivated varieties in Montenegro are with white flesh, mainly variety Kennebec. Its share in the total area under potatoes until recently accounted for a whopping 60% (Jovović et al., 2013).Although Montenegro is a traditional market of white flesh varieties, there is an evident trend of increased production of yellow flesh varieties in recent years. In addition, popularity of red skin varieties is gradually increasing in recent years as well (Jovović et al., 2013b). Although skin and flesh colour do not affect the quality of tubers, it significantly determines customer interest. Stability of flesh colour after cooking significantly affects its marketability (Momirović et al., 2000).

Despite the extraordinary consumption quality, Kennebec is a very old variety, with a very low genetic yield potential, but also some other problems and it is no longer able to respond to modern trends in this production. All this imposes a totally different concept of development of potato production and increased demand for new varieties.

Some potato varieties have the ability to provide stable yield of tubers in very divergent environmental conditions and in longer terms (Annicchiarico, 2002). That is why the study of

different genotypes of potatoes is of great importance as it is the most reliable way to find varieties best adapted to given environmental conditions (Yang, 2002, and Jovović et al., 2012) able to consistently give high yields in a wide range of environmental factors (Haldavankar et al., 2009).
The aim of this study was that to estimate genetic potential of some new varieties of potato with white flesh colour and compare them with dominant cultivated variety Kennebec in the conditions of the mountain areas of Montenegro.

Materials and methods

The study of productivity of five Dutch white flesh potato varieties was done in 2013 and 2014. Following varieties were studied: Arrow (very early variety) and Messi, Volare and Dido (medium early varieties). Variety Kennebec was used as a standard. The experiments were conducted in Nikšić, at an altitude of 800 m, on acid brown soil.
The study was carried out using field trials in a randomized block design with 4 replications. The plot size was 21 m^2. Planting of potatoes was done manually with 70 cm between row distance and 33 cm within row plant distance respectively, achieving the density of 43300 plants per hectare. Standard agricultural practice for the potato crop was applied. Potato harvesting was done after full maturation of canopy. The potato yield in the experiment was determined by measuring the tubers at each elementary plot, and then the yield per hectare was calculated.

Table 1. Chemical characteristics soil on experimental field

Depth (cm)	Locality	pH		$CaCO_3$	Humus	Soluble mg/100 g	
		H_2O	nKCl	%	%	P_2O_5	K_2O
40	Nikšić	6.57	5.97	1.75	3.05	9.3	17.3

Soil on which the experiments were carried out is characterized by favourable water and air properties and high humus content. This soil is poor in phosphorus and calcium and with moderate potassium content (Table 1).Meteorological data is given in Table 2.

Table 2. Meteorological conditions during the experiments

Locality	Year	Month					Average
		April	May	June	July	August	
Air temperature (^{0}C)							
Nikšić	2013	12,2	14,7	18,8	22,6	23,4	18,3
	2014	10,2	13,7	18,7	19,8	21,0	16,7
	Amount of rainfall (mm)						Total
	2013	194,1	145,0	58,3	36,3	57,7	491,4
	2014	151,2	115,4	165,3	187,7	17,6	637,2

Results and discussion

The results of investigation given in Table 3 show that the largest number of tubers in this study was determined in the cultivar Dido -10, while the lowest number had variety Kennebec - 7. Variety Dido had significantly higher numbers of tubers compared with all other varieties. On the other hand, the variety Kennebec compared to other studied varieties gave lowest number of tubers.

Table 3. Results of the investigation

Parameter	Average tuber number			Average tuber weight (g)			Tuber yield (t.ha^{-1})		
Year (B)	2013	2014	Average (A)	2013	2014	Average (A)	2013	2014	Average (A)
Variety (A)									
Arrow	7.3	9.4	**8.4**	89.5	84.8	**87.2**	28.3	34.4	**31.4**
Messi	7.9	7.7	**7.8**	97.5	122.3	**109.9**	33.4	40.7	**37.1**
Volare	8.9	9.4	**9.2**	84.3	90.5	**87.4**	32.3	36.7	**34.5**
Dido	9.8	10.1	**10.0**	78.3	79.2	**78.8**	33.1	34.5	**33.8**
Kennebec	6.9	7.1	**7.0**	85.3	94.8	**90.1**	25.4	29.2	**27.3**
Average (B)	**8.2**	**8.7**		**87.0**	**94.3**		**30.5**	**35.1**	

	A		B		A*B	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01
Average tuber number	0.482	0.661	0.305	0.418	0.682	0.935
Average tuber weight (g)	5.669	7.775	3.585	4.917	8.017	10.995
Tuber yield (t.ha^{-1})	2.156	2.956	1.363	1.870	3.048	4.181

The average number of tubers is a very important parameter of potato productivity. It significantly depends on the number of primary stems formed on one potato plant. It influences the production per plant, and finally the total yield per unit area. According to Bugarić et al., (2000) the high and stable yields gave the varieties forming 10 tubers per plant. Tubers with the highest average weight were measured on variants where variety Messi (109.9 g) was planted. The smallest tubers were found in Dido variety (78.8 g). Analysis of variance of the average tuber weight showed a statistically highly significant increase in mass of the variety Messi compared with all other varieties. Differences in tuber sizes between Dido variety and all other applied treatments were also statistically significant. Tubers size, as well as their number is determined by the number of sprouts that are formed by a single plant. Size is important characteristic of tuber productivity and basically represents a varietal characteristic. However, it dominantly depends on the level of agricultural practices, soil type and weather conditions during the vegetation season. According to Butorac and Bolf (2000) and Jovović (2012b) the higher number of primary shoots gives the higher number of tubers formed, but not their weight, and vice versa. The results obtained are in favour to these conclusions.

Highest potato yield in two years study was obtained in variety Messi - 37.1 t.ha^{-1}, and lowest in Kennebec – 27.3 t.ha^{-1}. High potato yields had also varieties Volare and Dido (34.5 and 33.8 t.ha^{-1}, respectively). Differences in yields obtained between variety Messi and all other varieties were statistically significant. Also, all varieties gave significantly higher tuber yield compared to Kennebec.

In the two years studies, higher average yields of potatoes were measured in 2014 – 35.1 t.ha^{-1} (average yield of potato in 2013 was 30.5 t.ha^{-1}). Among the studied years statistically significant differences in the average tuber yield was found. These results are explained by higher precipitation in 2014 (637.2 mm) and a better distribution of monthly rainfall. Lower rainfall in 2013 in June and July (58.3 and 36.3 mm, respectively) contributed to these results. Given the relatively low average potato yields in Montenegro achieved yields can be considered quite high.

Conclusion

The two-year research results of investigation of productivity of different Dutch white flesh potato varieties in mountainous region of Montenegro allow us to conclude:

All studied varieties of potato had significantly higher tuber yield in comparison with the variety Kennebec that was used as a standard in these studies.

Bearing in mind obtained tuber yields, all four studied white flesh potato varieties can be very reliable substitute to still dominantly cultivated variety Kennebec.

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CONTENT OF MANGANESE, COPPER AND ZINC IN ALFALFA AND GRASSES IN RELATION TO THE SOIL PROPERTIES IN SERBIA

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Abstract

The aim of our research was to investigate the content of Mn, Cu and Zn in soil and their effects on mineral uptake and quality of alfalfa and grasses. During 2013 soil (n=28) and plant (n=28) samples from 15 different localities on the territory of Vojvodina province and West Serbia were collected. Soils from these sites can be classified in four soil types: chernozem, fluvisol, arenosol and pseudogley. The total and available (DTPA extracted) concentrations of Mn, Cu and Zn were analyzed, as well as basic soil properties. The above plant mass concentrations of Mn, Cu and Zn were determined and correlated to soil concentration of the analyzed elements and soil properties. The results have shown that most of soils were well supplied with Mn and Cu. However, from the aspects of ruminant nutrition, on some locations plants were not sufficiently provided with these microelements. The concentration of available Zn in soil were sufficient on almost all locations (1.6 mg kg^{-1} under alfalfa and 2.1 mg kg^{-1} under grassland), but analyzes of plant material showed that Zn level was very low in all collected alfalfa (9.68 mg kg^{-1}) and grass samples (8.31 mg kg^{-1}). Thus, certain agricultural methods should be applied in order to increase Zn content in plants used for animal feed.

Key words: *manganese, copper, zinc, alfalfa, grasses*

Introduction

Manganese, copper and zinc are recognized as essential for optimum growth and reproduction of most crops, but they are also vital for livestock and humans (Gupta and Gupta, 2005). For crops, soil is the main source of these elements, but only a small part of the total content is available to plants. The mobility and availability of trace elements are controlled by many chemical and biochemical processes such as precipitation-dissolution, adsorption-desorption, complexation-dissociation, and oxidation-reduction (He et al., 2005).

The soil plant transfer of trace elements is part of a complex chemical element cycle in the nature. The most important factors that affect the availability of trace (micro) elements are: pH and redox potential, soil texture, organic matter-quantity and quality, mineral composition, temperature and water regime, as well as interaction between the chemical elements (Kebata-Pendias, 2004). In their study, Lombnaes and Singh (2003) report that the inclusion of soil parameters like clay content and total concentration in soil, in addition to soil extraction data represent the tool for improving models for simple determination of phytoavailable Zn and Cu.

The interest to quantify the levels of trace elements in cattle forage lies in the importance of micronutrients for animal health and the connection between the quality of forage and the nutritional aspects of cattle health (Moscuzza et al., 2012).

Copper is an important microelement for normal functioning of numerous processes in the animal organism. It is required as a catalytic cofactor of selective oxidoreductases and it is important for ATP synthesis, normal brain development and neurological function, immune

system integrity, cardiovascular health, and bone density in elderly adults (Barker and Pilbeam, 2006).
Manganese plays an essential role in photosynthesis and in catalysing the conversion of free oxygen radicals to hydrogenperoxide as part of the enzyme superoxide dismutase, due to its capability to easily change oxidation states (Hooda, 2010). Manganese deficiency during pregnancy could affect the offspring as irreversible incoordination of muscles, that leads to uncontrolled movements by an animal (Barker and Pilbeam, 2006).
Zinc is a microelement required in low but necesarry quantities and if its available content is not appropriate, plant and animals will suffer from different physiological stress. These stresses are caused by dysfunctionality of several enzyme systems and various disorders of metabolic functions in which zinc has an important role (Alloway, 2008).
The aim of the research was to examine the content of Mn, Cu and Zn in soil, as well as other soil properties and their effects on the uptake and quality of alfalfa and grasses.

Material and method

During 2013 soil and plant samples were collected from 15 different locations on the territory of the Vojvodina province and West Serbia (Kolubara county). From the selected sites, soil samples were taken from under the grassland (n=15) and alfalfa (n=13), as well as appropriate plant material. Soils from these sites can be classified in four soil types: chernozem, fluvisol, arenosol and pseudogley.
Soil samples were collected from the 0-30 cm depth. The pH was measured in a ratio 1:2.5 soil:water and soil:1M KCl suspension. The $CaCO_3$ content was determinated volumetrically using Scheibler calcimetar. The total C content was determinated by the Tjurin method. Available phosphorus and potassium were extracted using the ammonium lactate solution (Egner and Riehm, 1960). The content of phosphorus was determinated by spectrometery and potassium by flame photometer. The total concentrations (pseudo total) of Cu, Mn and Zn were determinated after digestion with HNO_3 and H_2O_2 and the concentration of available microelements in soil after the extraction using the DTPA solution. After digestion or extraction of elements, concentrations were measured by the atomic adsorption spectrophotometer (Shimadzu 6300), with flame technique.
Forage samples were taken at the beginning of May 2013. The above-ground plant mass was collected randomly from the same fields as the soil samples. The samples were digested with a mixture HNO_3:$HClO_4$ and the concentrations of Mn, Cu and Zn were determinated by the atomic adsorpiton spectrometer (flame technique).
The data obtained in the study were processed by the correlation-regression analyses. A statistical analysis was performed by a statistical software STATISTICA 12.

Results and discussion

Table 1 shows the mean value and range of basic properties of soil from different locations in Serbia. On the localities in Vojvodina, soils are with neutral to alkaline reaction, medim to extremely calcareous. On the localities in West Serbia, because the soil type is pseudogley, the soil reaction is extremely acid under the grassland and slightly acid under the alfalfa, with a low content of $CaCO_3$. The average concentrationsof plant available phosphorus and potassium under alfalfa and grasses were on optimum level for plant production. Only on few locations soils were insufficiently rich with these elements.
The world's soils average concentration of Cu is 20 mg kg^{-1}, while Zn varies in the range of 10-300 mg kg^{-1} (Xie and Lu, 2000). Comparing the data presented in Table 2, the Cu concentration was slightly below the world’s average, while the average concentration of Zn corresponds to the world's average. Also, in comparison to previous studies in Serbia, the content of copper is similar to the average results in Vojvodina (17,1 mg kg^{-1}) (Ubavić et al.

1993), while the Zn content varies considerably in relation to the average (60.3 mg kg^{-1}). The average value of the measured total Mn in soil under grassland (629.82 mg kg^{-1}) and alfalfa (610.09 mg kg^{-1}) are above the world's soil mean of 437 mg kg^{-1}(Kebata-Pendias, 2000).

Table 1. Mean and range of basic soil properties

	Soil property					
	pH		Percent		mg $100g^{-1}$	
	H_2O	**KCl**	**$CaCO_3$**	**Humus**	**P_2O_5**	**K_2O**
Soil under grassland						
Mean±SD	7.66 ± 1.18	6.69 ± 1.25	9.69 ± 8.44	2.62 ± 0.72	23.44 ± 18.99	20.87 ± 11.96
Range	5.3-9.4	3.88-7.83	2.49-26.79	1.78-4.62	0.85-57.13	6.50-46.07
Soil under alfalfa						
Mean±SD	7.79 ± 0.89	6.91 ± 1.06	7.93 ± 5.13	2.32 ± 0.71	17.75 ± 11.75	16.65 ± 5.48
Range	5.52-8.37	5.27-7.64	2.08-18.99	1.0-3.22	0.21-41.36	7.29-26.58

In all soil samples the concentrations of available Cu and Mn are above critical concentration of 0.2 mg $kg^{-1}$and 1 mg kg^{-1}, respectively (Lindsay and Norvell, 1978) and therefore at an adequate level to satisfy plant nutrition. Out of 28 analyzed samples, at two sites concentration of available Zn were insufficient, less than 1 mg kg^{-1}.

Table 2. Mean and range of total and available content of the observed microelements

	Micronutrient status (mg kg^{-1}) in the soil					
	Manganese (Mn)		**Copper (Cu)**		**Zinc (Zn)**	
	Total	Available	Total	Available	Total	Available
Soil under grassland						
Mean± SD	629.8±249.3	29.6±32.4	18.7±5.3	8.0±3.0	25.9±6.0	2.1±0.8
Range	232.8-1058.3	1.7-95.8	11.1-37.8	4.4-16.0	19.7-41.2	0.9-4.4
Soil under alfalfa						
Mean± SD	610.1±270.4	29.35±22.93	17.8±6.2	10.9-30.8	25.6±10.0	1.6±0.9
Range	201.0-1193.0	4.82-107.65	6.2±2.3	3.4-12.1	13.8-52.0	0.02-3.8

In previous studies in Vojvodina, measured concentration of Mn, Cu and Zn in alfalfa samples in were 0.1-46 mg kg^{-1}, 0.1-8.3mg kg^{-1} and 0.08-26.3 mg kg^{-1}, respectively.(Čuvardić et al., cited by Manojlović and Singh, 2012). Comparing with the date in Table 3, concentration of Mn and Cu in few alfalfa samples were higher than the defined range. In Serbia, concentration of copper in grass samples varies from 2-12.2 mg kg^{-1}(Danon and Jakoviljević, cited by Manojlović and Singh, 2012). In more then 26% grass sample collected in this research, Cu concentration was higher than 12.2 mg kg^{-1}.

Tabel 3.Mean and average value of Mn, Cu and Zn content in grasses and alfalfa

Micronutrient status in grasses and alfalfa (mg kg^{-1} on dry matter basis)					
Manganese (Mn)		**Copper (Cu)**		**Zinc (Zn)**	
Mean± SD	Range	Mean± SD	Range	Mean± SD	Range
Grasses					
82.59±49.37	25.85-214.79	7.03±1.78	4.74-12.00	8.31±1.70	5.11-11.46
Alfalfa					
38.48±10.14	19.87-53.58	8.54±2.74	0.44-11.93	9.68±1.77	6.55-13.07
Sufficiency levels in livestock feeds (Gupta and Gupta, cit. Manojlović and Singh, 2012)					
Ruminants : 40 mg kg^{-1}		Sheep: 6-10 mg kg^{-1}		Dairy cattle and Bulls :50 mg kg^{-1}	

Frequently, Cu, Mn and Zn levels in crops that are sufficient for optimum crop yield are not adequate to meet the needs of livestock animals (Gupta and Gupta, 2005). The data given in Table 3. in comparison to sufficiency levels for various classes of livestock, has shown that in average, grasses and alfalfa are sufficiently provided with Mn and Cu. However at few sites concentration of these microelements in plant material are lower than the ruminants requirements.

Table 4.Correlation coefficients (R) between soil parameters and plant concentrations (alfalfa+grasses)

Correlation coefficients (R) between soil parameters and plant (alfalfa+grasses)							
	Plant				Plant		
	Mn	**Cu**	**Zn**		**Mn**	**Cu**	**Zn**
General soil parameters				**Micronutrient soil parameters**			
Humus	-0.110	0.071	-0.080	Total Mn	0.313	-0.051	0.123
$CaCO_3$	0.018	-0.260	-0.110	Av. Mn	**0.484**	-0.190	0.025
P_2O_5	-0.090	-0.024	0.038	Total Cu	**-0.390**	0.237	0.020
K_2O	-0.280	0.349	0.160	Av. Cu	-0.120	0.162	-0.070
pH in H_2O	-0.320	-0.120	-0.360	Total Zn	-0.230	0.166	0.217
pH in KCl	-0.310	-0.120	**-0.390**	Av. Zn	-0.080	-0.250	0.165
			Manganese in plants		x	-0.290	-0.090
			Copper in plants			x	0.236

*Correlation in bold is significant at the 0,05 level

Although soils in almost all locations were well-provided with available Zn, the results have shown that plant content is considerably below cattle needs of 50 mg kg^{-1}. Lindsay (1972) listed nine major factors affecting zinc availability (and hence likely to be related to the deficiency) which included: soils with low zinc content, soil with a restricted root zone, calcareous soils, low organic matter, microbiologically inactive zinc, cool soil temperatures, plant species and varieties, high levels of available phosphorus, and the effect of nitrogen.
Table 4 presents correlations coefficients betwen basic soil properties and the content of Mn, Cu and Zn in grasses and alfalfa, as well as the correlations among the total and available content in soil and the content of these elements in plant material.
Significant correlation was found between Mn content in plants (grasses and alfalfa) and the concentration of available Mn (R=0.484) and total Cu in soil (R=-0.390), respectively. There was no significant correlation between soil properties and Cu and Zn content in plants, except significant negative correlation between Zn content in plants and pH in soil.

Conclusion

The average content of total copper in soil samples under grasses and alfalfa (18.7 mg kg^{-1} and 17.8 mg kg^{-1}, respectively) was slightly lower, while the total concentration of manganese (629 mg kg^{-1} and 610 mg kg^{-1}) is higher in comparison to the world's soil average. The zinc concentration corresponds to the world’s average. Most of the examined soils are well-supplied with available Mn and Cu and from the aspects of ruminant nutrition, plants were insufficiently stocked only in few locations. The concentration of available Zn in soil was sufficient in almost all locations (2.1 mg kg^{-1} under grassland and 1.6 mg kg^{-1} under alfalfa), but Zn content in alfalfa (9.68 mg kg^{-1}) and grasses (8.31 mg kg^{-1}) was very low in comparison to dairy cattle needs. Thus, certain agricultural methods should be applied in order to increase Zn content in plants used for animal feed. There was no significant

correlation between the observed soil parameters and the content of Mn, Cu and Zn in grasses and alfalfa, except few correlation in case of Mn and Zn. Apart from the observed soil characteristics, other soil factors should be included in modeling phytoavailable microelements to grasses and alfalfa.

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SPATIAL ANALYSIS OF TEMPORAL TRENDS IN GROWING SEASON LENGTH FOR SERBIA OVER THE PERIOD 1961–2010

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Abstract

The study aimed to evaluate spatiotemporal changes of growing season length (GSL) in response to observed warming in Serbia for the period 1961–2010, using temperature observations from 26 meteorological stations uniformly distributed over the country. GSL was climatologically defined as the number of days between the first span of at least 6 days with Tmean > 5°C and the first occurrence after 1 July of at least 6 consecutive days with Tmean < 5°C. Temporal trends were evaluated by a least-squares linear regression method for each station and for the entire Serbian territory using the average series. Trends were assessed for data spanning the 1961–2010 and 1986–2010 time intervals. It was found that GSL had increased in Serbia, with the greater magnitude in the recent-past. Nationally averaged rate of change was 5.1 days/decade for the entire period and 14.8 days/decade for the period 1986–2010. The greatest increase in GSL was observed at Negotin, with overall trend of 9.7 days/decade and change of 26.7 days/decade over the second half of the observation period. Shortening of growing season was observed only at Pozega, with trends of –1.3 days/decade for the entire period and 3.8 days/decade for the period 1986–2010. In addition, the relationship between the GSL and large-scale circulation features was examined. It was found that the inter-annual variation of GSL was significantly correlated with the East Atlantic (EA) pattern.

Key words: *growing season length, climate change, Serbia*

Intorduction

The globally averaged surface air temperature increased by 0.85°C (0.65–1.06°C) over the period 1880–2012, according to the IPCC (Intergovernmental Panel on Climate Change) Fifth Assessment Report (IPCC, 2013). Most of the warming occurred from 1951 to 2012, with warming rate of 0.12°C per decade. In association with this global warming, the growing season (GS) start, end and length have changed over most of the Northern Hemisphere during the late 20th century (e.g. Pamersan and Yohe, 2003; Linderholm, 2006; Jeong et al., 2011). Extension of GS was mainly attributed to earlier start of GS (e.g. Menzel and Fabian, 1999; Linderholm et al., 2007; Gordo and Sanz, 2010; Irannezhad and Kløve, 2015). Changes in the timing and length of GS may have profound impacts on plant and animal ecosystems (e.g. White et al., 1999; Root et al., 2003; Piao et al., 2007).

There is no universal definition of GS. It can be phenologically defined (e.g. Menzel and Fabian, 1999) and based on developmental stages, such as bud-burst, flowering, leaf unfolding (determining the start of GS), and leaf coloring and leaf falling (determining the end of GS). Since phenological data are scarce in many parts of the world, the normalized difference vegetation index (NDVI) obtained from satellite data is commonly used indicator of phenological dynamics on larger scales (e.g. Tucker et al., 2001). The GS can be also climatologically defined using different thresholds related to climatic conditions in particular area (e.g. Irannezhad and Kløve, 2015). For instance, it can be defined as the period between the dates of last spring frost and first autumn frost. The period with daily mean temperature

(Tmean) greater than 5°C is usually considered as GS for most crops grown in temperate zone. A number of studies based on satellite, meteorological and phenological observations showed evidence of a lengthening of GS in different parts of the world (Linderholm, 2006).
Inter-annual variation in GS has been linked to large-scale atmospheric phenomena in many studies (e.g. Jones et al., 2002; Menzel, 2003; Aasa et al., 2004; Irannezhad and Kløve, 2015). Large-scale atmospheric circulation patterns describe long-term deviations in natural occurrence of chaotic atmosphere behavior. Dominant atmospheric teleconnection patterns in the part of Europe where Serbia is located are North Atlantic Oscillation (NAO), East Atlantic (EA) pattern (EA) and East Atlantic/West Russia (EA/WR) pattern.
This study aimed to spatially analyze historical trends in climatologically defined growing season length (GSL) in Serbia and explore relationships between large-scale atmospheric circulation patterns and GSL.

Materials and methods

Temperature data were provided by the Republic Hydrometeorological Service of Serbia for 26 meteorological stations, quite uniformly distributed over the Serbian territory. Recent data were not available for the Autonomous Province of Kosovo and Metohija situated in the southwestern part of Serbia (Figure 1). The acronym, name, geographical location and altitude of selected station are given in Table 1.
In this study, GSL was defined as the number of days between the first span of at least 6 days with Tmean > 5°C and the first occurrence after 1 July of at least 6 consecutive days with Tmean < 5°C. Temporal trends were assessed by a least-squares linear regression method for each station and for the entire Serbian territory using the average series. The statistical significance of the change was determined using a *t*-test. Trends were calculated for the entire observational period form 1961 to 2010 and for the recent half of the period from 1986 to 2010. Pearson correlation coefficient was computed in order to investigate relationships between the large-scale atmospheric circulation patterns and GSL. The pattern index values were downloaded from the NOAA Climate Prediction Center website (ftp://ftp.cpc.ncep.noaa.gov/wd52dg/data/indices/tele_index.nh).

Results and discussion

The mean GSL and decadal trends for each station over the periods 1961–2010 and 1986–2010 are shown in Table 1. Belgrade was the station with the longest GS over both periods, followed by Kragujevac considering the entire observation period, and by Smederevska Palanka considering recent decades. The shortest GS was observed at the highest stations Sjenica and Zlatibor over both periods. The mean GSL averaged across all station was 267.8 days for 1961–2010 and 274.7 days for 1986–2010. The extension of GS was more pronounced in the more recent period. The greatest increase of GSL after 1985 was observed at Negotin (26.7 days/decade) and Dimitrovgrad (25.9 days/decade) and the smallest at Pozega (3.8 days/decade). The largest trend in GSL over the entire period was recorded at Negotin (9.7 days/decade) and Zajecar (9.1 days/decade), while the smallest, even negataive trend was observed at Pozega (–1.3 days/decade). On average for all station, the increase of GSL was 14.8 days per decade over the last 25 years of the observation period and 5.1 days per decade for the entire period (Fig. 1). Spatial variability displayed for GSL trends had different patterns for two periods (Fig. 2). This can be explained by a decreasing trend in GSL over the first half of examined period at some stations, which resulted in a decreasing trend in GSL averaged across all stations over the same period (Fig. 1). The decreasing trend in GSL at the beginning of observation period can also explain negative overall trend at Pozega, despite increasing trend over the second half of examined period. Dimitrovgrad, having the second highest trend since 1985, was among stations with the smallest overall trend and had

nearly the same GSL over two examined periods (Table 1) for the same reason – a negative trend in GSL at the beginning of observation period. The similar pattern of change, due to cooling trend, was observed at Vranje.

Table 1. Geographical position and elevation of meteorological stations used in the study, mean growing season length (GSL) and decadal trends in GSL for the periods 1961–2010 and 1986–2010.

Code	Name	Latitude (°N)	Longitude (°E)	Elevation (m)	Mean GSL		Trend (days/decade)	
					1961–2010	1986–2010	1961–2010	1986–2010
BG	Beograd	44.80	20.47	132	287	297	6.5*	2.2
CU	Cuprija	43.93	21.37	123	271	279	4.9	11.1
DM	Dimitrovgrad	43.02	22.75	450	264	265	3.4	25.9**
KI	Kikinda	45.85	20.47	81	264	271	4.6	10.8
KG	Kragujevac	44.03	20.93	185	284	289	5.4	11.1
KV	Kraljevo	43.72	20.70	215	271	278	5.5*	18.3*
KS	Krusevac	43.57	21.35	166	276	282	6.2*	21.5*
KU	Kursumlija	43.13	21.27	383	273	281	6.6*	20.5*
LE	Leksovac	42.98	21.95	230	272	275	4.0	18.5*
LO	Loznica	44.55	19.23	121	281	290	5.7*	6.9
NE	Negotin	44.23	22.55	42	267	278	9.7***	26.7***
NI	Nis	43.33	21.90	204	281	286	5.0	17.3
PA	Palic	46.10	19.77	102	261	266	3.6	10.7
PZ	Pozega	43.83	20.03	310	253	252	-1.3	3.8
RS	Rimski Sancevi	45.33	19.85	86	271	279	5.5*	11.9
SJ	Sjenica	43.27	20.00	1038	223	227	3.5	11.5
SP	S. Palanka	44.37	20.95	121	276	286	6.3*	8.0
SO	Sombor	45.77	19.15	87	266	276	6.8**	13.1
SM	S. Mitrovica	45.02	19.55	82	270	276	5.6*	17.1*
VA	Valjevo	45.77	19.15	87	276	285	4.9	5.2
VG	V. Gradiste	44.75	21.52	80	266	274	3.6	6.2
VR	Vranje	42.55	21.92	432	272	271	1.2	17.2*
VS	Vrsac	45.15	21.32	84	277	286	5.6*	7.6
ZA	Zajecar	43.88	22.28	144	259	274	9.1***	12.9
ZL	Zlatibor	43.73	19.72	1028	234	241	6.0*	11.0
ZR	Zrenjanin	45.40	20.38	80	270	279	5.8*	9.6

*Statistically significant at the 0.05 level;
**Statistically significant at the 0.01 level;
***Statistically significant at the 0.001 level.

Obtained trend of GSL in Serbia over the period 1961–2010 is in line with earlier results of extension of GS in the Northern Hemisphere, even though been somewhat greater than those reported in previous studies. Menzel et al. (2003) found a lengthening of the climatological GS in Germany during the period 1951–2000, with change rate of 0.11–0.49 days/year depending on the definition used. According to Liu et al (2010), the GSL in China increased by 6.9–8.7 days over the period 1951–2000, depending on the base temperature chosen. Irannezhad and Kløve (2015) reported an increase of GS by 0.30 days/year on national scale in Finland for the period 1961–2011, for GS defined as the period with mean daily temperature permanently exceeding 5°C.

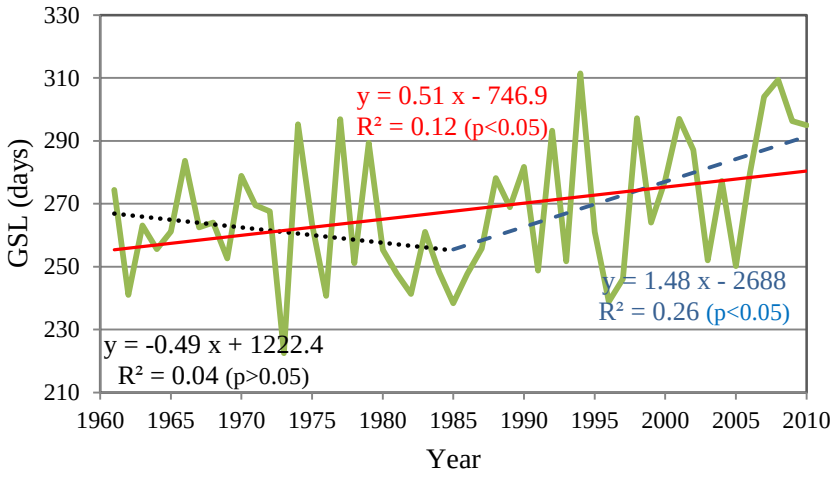

Figure 1. Inter-annual variation of growing season length (GSL) averaged over 26 stations during 1961–2010. The red solid, black dotted and blue dashed lines represent best-fit linear regressions for the periods 1961–2010, 1961–1985 and 1986–2010, respectively.

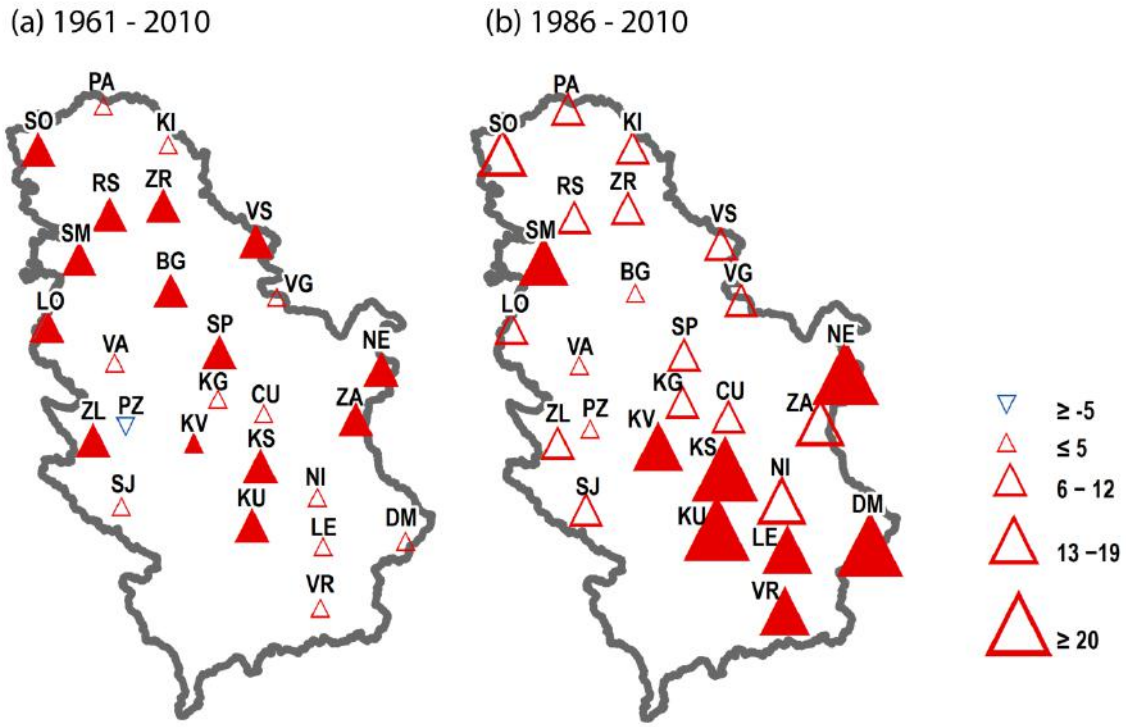

Figure 2. Spatial distribution of trends in growing season length (days/decade) in Serbia over the periods (a) 1961–2010 and (b) 1985–2010. Upward and downward pointing triangles show increasing and decreasing trends, respectively. The filled triangles correspond to statistically significant trends at the 0.05 level.

Results of correlation analysis showed that GSL was significantly correlated only with EA pattern (Fig. 3), considered to be the second dominant teleconnection pattern across the North Atlantic. The positive phase of the EA pattern is associated with above-average temperatures in Europe throughout the year. Significant correlation found between the EA and GSL variability suggests that global warming may not be the only explanation for extension of GS in Serbia.

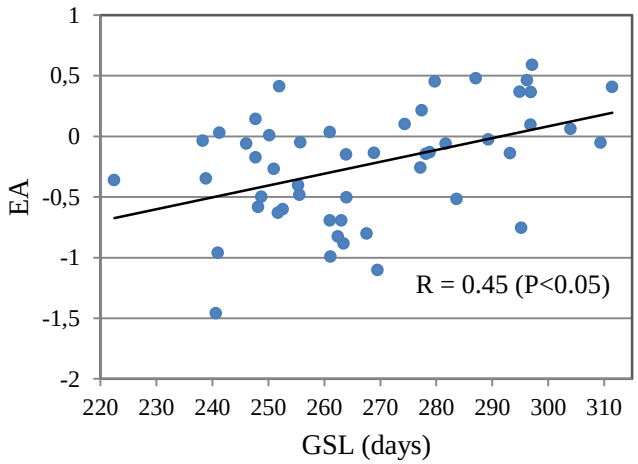

Figure 3. Scatter plot of correlation between growing season length (GSL) and most significant associated large scale circulation pattern East Atlantic (EA).

Conclusion

This study examined trend in GSL and the link to large-scale circulation patterns, using long-term daily mean temperature dataset for the 26 meteorological stations across Serbia. On average for all stations, GSL significantly increased by 5.1 days per decade during the period 1961–2010 and by 14.8 days per decade during the period 1986–2010. Increase in GSL during recent decades was most pronounced in the southeastern part of the country, with the greatest trend observed at Negotin. The correlation analysis revealed that the East Atlantic Pattern (EA) had much stronger association with annual temperature indices than he North Atlantic Oscillation (NAO) and East Atlantic/West Russia Pattern (EA/WR).

Observed lengthening of GS may enhance the potential for crop production and increase crop yields, but may also cause an invasion of weedy and other mobile species, and disturbance of species interaction such as those between plants and pollinators.

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THE EFFECTS OF DIFFERENT COVER CROPS ON FLORISTIC COMPOSITION OF WEEDS IN SWEET MAIZE

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Abstract

Weed management is a major issue in sustainable farming systems. The growing of cover crops is one alternative to herbicides, but using cover crops for weed control without adverse effects on main crop growth is important. The objective of this study was to determine the effect of winter grown cover crops (common vetch, oat and fodder kale) on floristic composition of weed community in sweet maize. The cover crops were sown in two growing seasons on slightly calcareous chernozem (the locality of Zemun Polje) after winter wheat as a previous crop. Plots without cover crops were used as a control: dead organic mulch-soil covered with straw in autumn and winter time, and conventional variant-bare soil uncovered during autumn and winter time. Mowing of the above-ground biomass of cover crops was performed 7-10 days before planting of sweet maize. The estimation of weed infestation in sweet maize was conducted in early July. The average number of species of weeds in sweet maize was from 7.1 and 7.9 (common vetch and dead organic mulch) across 7.8 and 9.6 (fodder kale and conventional variant) to 13.1 (oat). The number of weed plants was also higher in the variant cover crop with oat. However, aboveground fresh and dry weights of weeds had the highest value in control variant in both investigation years. It can be concluded that perennial weeds could be controlled effectively by sowing cover crops in sustainable farming systems in a semi-arid regions.

Key words: *cover crops, dead organic mulch, sweet maize, weed infestation*

Introduction

On the infestation of a crop affect a large number of factors, especially cropping system, weather conditions, soil properties and type of cultivated crops. If a crop is grown in the sustainable and organic agriculture, the problem of weed control is more pronounced. Investigations conducted in countries around the world point to greater diversity of weed community, larger population and higher biomass weed production in these systems compared to conventional production. The most trusted extent of weed control in sustainable and organic agriculture systems is alternative measures and increasing biodiversity in agro-ecosystem. Cultivation of winter cover crops (dead mulch) or spring cover crop (living mulch), in terms of weed control, does not have an alternative. There are potential benefits from winter cover crops growing: nutrient enhancement, soil nutrient capture, soil moisture retention, erosion protection, improved soil structure, disease control, nematode control, increased soil organic matter, etc. But, there are some risks of winter cover crops: additional management and labour, additional expense for seed cost, interference with primary crop establishment, soil moisture depletion (if cover crop actively growing in spring), cooler soil temperatures in spring because of plants on surface, competition with a primary crop, nutrient depletion by non-legumes, nutrient availability not timely for subsequent crop, allelopathic effects on primary crop, etc. Cover crops help control weeds in spring and fall by out-competing them for resources, by not allowing a niche for them to germinate and through

allelopathic compounds. Be aware that all weeds and all weed species will not be controlled, even under ideal cover crop growth. Season-long weed control cannot be expected; early season control of weeds is more likely. Small seeded annual weeds are controlled more than other weeds by cover crops. Cover crop residue can have allelopathic effects that inhibit the germination of some weed species. However, this effect will be more efficient with high amounts of residue.

Weeds in sweet maize, especially in the initial period of growth, can cause significant reductions in crop growth and can cause a decrease in yields of ear by up to 85% (Williams, 2010). Concern about significant weed growth by not using herbicides has limited the adoption of organic methods, such as cover cropping and mulching (Bond and Grundy, 2001). Cover crops have been utilised to reduce weed management costs, as well as to enhance soil characteristics (Fageria et al., 2005). Some producers of herbicides indicate their phytotoxic influence on the sweet maize plants. In these situations, efficacy of mowed or desiccated mulch to manage weeds seems to depend most on soil coverage and its effect on light interception, rather than on the effects of altered moisture or temperature regimes, allelopathy or mechanical impedance (Liebman and Davis, 2000). Ground cover greater than 50% results in a roughly exponential increase in weed suppression.

Winter cover crops are best adapted to areas with a long enough time period to establish in the fall and without soil moisture deficits in the spring. The first step in selecting a cover crop species is to determine the main goal of the cover crop. Many organic producers select cover crops to add nitrogen, control weeds, protect soil, and/or to increase soil organic matter. There are two main categories to consider cover crops that over-winter and regrow in the spring, and those that do not. In northern climates, many cover crop species will not survive the winter. Winter rye and hairy vetch are cover crops that have the best potential to overwinter. Oat planted in the fall is an example of a cover crop that will winter kill. Producers will need to choose if overwintering is a desirable winter cover crop characteristic. Leguminous cover crops will provide nitrogen to subsequent crops. This nitrogen can increase yield in sweet maize. Red clover and hairy vetch are the best choices.

Boydston and Al-Khatib (2006) reviewed *Brassica* cover crops and weed management. They focused on allelopathy as the mechanism responsible for weed suppression and the hydrolysis products of glucosinolates as the source of allelochemicals involved. Glucosinolates are secondary plant metabolites which have demonstrated a biological activity against weed seeds, pathogens, insects, and nematodes (Chew, 1988).

Paper aimed at comparing the effects of different cover crops systems with those of conventional weed management methods for weed suppression of sweet maize.

Material and Methods

Two-year trials were carried out on chernozem in the experiment field of the Maize Research Institute, Zemun Polje, in the vicinity of Belgrade (44°52'N 20°20'E). The soil was slightly calcareous chernozem with 47% clay and silt, and 53% of sand. The soil properties in layer 0–30-cm depth were fallow: 3.22% organic matter, 0.19% total N, 1.9% organic C, 16.2 and 22.4 mg per 100 g soil of available P_2O_5 and extractable K_2O, respectively, 1.38% total $CaCO_3$ and pH 7.3. The four-replicate plot was set up according to the randomised block design. The elementary plot size amounted to 16.8 m^2 (2.8 m by 6.0 m). The following factors were included into the studies performed under rain fed conditions:

(A) – different species of cover crops

(B) – year investigation.

The experiment included three kinds of *winter cover crops* (common vetch, oat and fodder kale), another variant in which the land was covered with dead organic mulch, and traditional

variant, classical ploughing in the fall and keeping bare land uncovered during the winter. All of the varieties being used as cover crops belong to Novi Sad Field Crops Institute.
Winter wheat was a preceding crop. The experimental plots being ploughed in the autumn have followed one pass of a disk harrow and a field cultivator prior to sowing. Sowing of cover crops was done manually in October 14, 2011 and November 02, 2012. Fallowing nitrogen fixation rates in legume crops, as well recommended fertilization, we came up to the required amount of macronutrients for sweet maize (120 kg ha^{-1} N, 90 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O). In the fall period, before planting of cover crops, we have entered the entire amount of P and K in the forms of monopotassium phosphate plus additional quantity of nitrogen 50 kg ha^{-1} by ammonium nitrate, and on the two control variants, also all of P_2O_5 i K_2O and 40 kg ha^{-1} N in the form of AN. In the next spring (April 04, 2012 and April 09, 2013), leguminous cover crop had received another 30 kg ha^{-1} N in the form of AN (remaining 40 kg ha^{-1} considered to be provided by nitrogen fixation), oats an fodder kale 70 kg ha^{-1} N, and control plots another 80 kg ha^{-1} N, also in the form of AN.

Mowing the above-ground biomass of winter cover crops was performed 7-10 days before planting or sowing of sweet maize. Sweet maize was sown in density of 65.000 plants ha^{-1}. The inter-row distance was 70 cm, while within-row plant distance was 22 cm. The new Zemun Polje (ZP) sweet maize hybrids ZP 441su (FAO maturity group 400) was sown. Planting of sweet maize seedlings was done on May 21, 2012, and sowing was done on April 25, 2013.

The floristic composition, number of species, number of plants per species, fresh and air dried weed biomass in sweet maize were analysed in this study. All stated parameters in weeds were determined by the one square meter area method. The weed infestation estimation was performed on July 10, 2012 and July 04, 2013. The estimation time was determined on the basis of the actual crop performance that was particularly affected by weather conditions during the years of investigation. Following the estimation, hoeing was done with the aim of suppressing the weeds in cover crops.
Obtained data were statistically processed by the analysis of variance, in which plant cover crops and years of investigation were factors, while LSD test was applied for the individual comparisons (Gomez and Gomez, 1984).

Meteorological conditions

Table 1. Average air temperatures and precipitation sums from April to September at Zemun Polje

Months	Temperature (°C)		Precipitation (mm)	
	2012	2013	2012	2013
April	14.4	14.9	67	22
May	17.9	19.8	128	104
June	24.6	21.7	14	49
July	27.1	24.1	39	3
August	26.2	25.4	4	44
September	22.0	17.3	31	59
Average/Sum	22.0	20.5	283	281

The meteorological conditions during the sweet maize growing season are presented in Table 1. In the growing season of 2012, there was a crop failure because of extreme drought and high temperatures. In the 2013 growing season was the similar in a total cumulative precipitation, but it was better distribution. The second year of study, regarding the meteorological conditions, was similar to the previous one and the difference was in slightly lower air temperatures at the beginning of the growing season of the crop and increased rainfall in July, which had a negative impact on yield, but not on the weediness of maize and soya bean.

However, precipitations in June were very important for sweet maize crop. Most precipitation occurred in May (104 mm), which had a significant influence on the initial growth of investigation crop and increasing competitiveness against weeds.

Results and discussion

Representative conclusions about the effects of different cover crops and meteorological conditions on the floristic composition, number of species, number of plant per species, fresh and air dried weight of weeds in sweet maize are obtained by the insight into the results shown in Tables 2, 3 and 4.

The most abundant weed species in the summer aspect of sweet maize were *Sorghum halepense* L. Pers., *Datura stramonium* L., *Chenopodium album* L., *Amaranthus hybridum* L., and *Solanum nigrum* L. and significant places in weed synusia were taken up by *Amaranthus retroflexus* L., *Amaranthus albus* L., *Convolvulus arvensis* L., and *Hibiscus trionum* L., which was also found in the same crop and locality (Simic et al., 2012).

Table 2. Weed floristic composition and number of weed plants m^{-2} of main crop (sweet maize) in 2012 (A)

Life forms	Weed species	Cropping system (B)				
		1	2	3	4	5
T	*Amaranthus retroflexus* L.	1.5		2.0		1.2
G	*Sorghum halepense* L. Pers.	2.8	3.5	6.0	3.0	6.7
T	*Solanum nigrum* L.	1.2	2.0	1.0	0.4	0.6
T	*Chenopodium album* L.	2.8	3.5	1.0	0.8	1.8
T	*Amaranthus hybridum* L.	2.0	1.7	2.3	1.0	2.2
T	*Hibiscus trionum* L.		0.5	0.5		1.0
G	*Convolvulus arvensis* L.	1.0	0.8	0.8	1.0	1.8
T	*Datura stramonium* L.	2.0	1.2	4.8	3.3	5.7
T	*Bylderdikia convolvulus* (L.) Dum.	1.3	0.8		1.3	0.8
T	*Portulaca oleracea* L.		0.5			
T	*Chenopodium hybridum* L.		2.3	1.3	0.5	1.3
T	*Amaranthus albus* L.		3.1	4.2		1.8
T	*Senecio vulgaris* L.	0.3	0.5			0.3
T	*Lactuca serriola* Torn.		1.3			
T	*Ambrosia artemisiifolia* L.		0.5		6.9	0.5
T	*Chamomilla recutita* (L.) Rauschert		0.3			
T	*Digitaria sanquinalis* (L.) Scop.		0.4		0.2	
Total number of weed species		9	16	10	10	13
Total number of plants per species		14.9	22.9	23.9	13.4	22.2
Number of annual weeds		7	14	8	8	11
Number of perennial weeds		2	2	2	2	2
Aboveground fresh weight of weeds (g m^{-2})		56.8	287.3	266.6	384.2	591.9
Aboveground dry weight of weeds (g m^{-2})		16.1	61.0	50.9	88.5	144.8

L.f.-life forms: T-therophytes, G-geophytes; 1-common vetch, 2-oat, 3-fodder kale, 4-dead organic mulch, 5-conventional treatment

Generally, the greater weed infestation can be observed in the first year of the investigation and the reason for the worse weather conditions, especially high air temperatures and unfavourable distribution of precipitation which corresponds to more resistant weed species. Differences in measured parameters of weediness depending on the year investigation were not statistically significant, except for the number of weed species (Table 5). The greatest number of weed species was declared in 2012, and most weed plants and the highest fresh weight of weeds owing to favourable weather conditions in 2013. Thus, favourable meteorological conditions are suitable for growth and development, both endangered, and all other species in agro ecosystems. The dead organic mulch (straw) is justified in sustainable systems of cultivation of sweet maize, primarily in terms of grain yield (Dolijanović et al.,

2012). However, from an infestation, especially perennial species, the advantage is on the side of growing cover crops (dead mulch). The number of species and number of plants per species in this variant is similar to the number in cover crops, but fresh weight mass shows us a strong presence of perennial weeds. In addition, on this plots, after the decomposition of organic matter, there is enough space and light for regular and vigorous weed growth.
If you look at the impact of investigation kinds of cover crop on the infestation of the main crops we notice that in both years the best result given common vetch, in terms of number of species, number of plants per species and especially in respect of fresh weed biomass per unit area (Tables 2 and 3). In all tested parameters, weeds in both investigation year significantly lowest values are obtained if the cover crop was common vetch. The next crop was winter fodder kale, while winter oat showed the weakest effect, especially in terms of the number of weed species and the number of plants per species. In earlier paper, it was emphasized that in terms of reducing the infestation of the main crops, oats gives the poorest results and generally it should be grown in mixtures with legumes (Dolijanović et al., 2013). The differences investigated parameters of weeds in sweet maize cultivated after cover crops, compared to traditional cropping system are generally statistically significant (Table 4).
On the investigated locality, alternative farming systems (intercrops and cover crops) showed efficacy in reducing weed infestation, particularly the number of plants per species and weed fresh weight per unit area (Dolijanović et al., 2007; 2008; 2013).

Table 3. Weed floristic composition and number of weed plants m^{-2} of main crop (sweet maize) in 2013 (A)

Life forms	Weed species	Cropping system (B)				
		1	2	3	4	5
T	*Amaranthus retroflexus* L.	0.8	0.5	1.8		
G	*Sorghum halepense* L. Pers.	2.3	2.5	5.5	3.3	5.5
T	*Solanum nigrum* L.	2.0	1.3	1.0	0.3	0.3
T	*Chenopodium album* L.	1.8	4.5	0.8	1.0	1.5
T	*Amaranthus hybridum* L.	1.3	3.5	1.0	0.8	0.3
T	*Hibiscus trionum* L.	0.3	0.3			
G	*Convolvulus arvensis* L.		0.5	1.0	0.5	1.5
T	*Datura stramonium* L.	2.3	1.5	3.8	2.3	6.8
T	*Bylderdikia convolvulus* (L.) Dumort	0.8	0.5		0.8	0.5
T	*Portulaca oleracea* L.		0.3			
T	*Chenopodium hybridum* L.		1.8	0.8	0.5	1.0
T	*Amaranthus albus* L.		2.8	3.8		1.8
T	*Senecio vulgaris* L.		0.5			0.3
T	*Lactuca serriola* Torn.		0.8			
T	*Ambrosia artemisiifolia* L.		0.3		6.5	0.3
T	*Chamomilla recutita* (L.) Rauschert		0.3			
T	*Digitaria sanquinalis* (L.) Scop.		0.3			
Total number of weed species		8	17	9	9	11
Total number of plants per species		11.6	22.2	19.5	16.0	20.8
Number of annual weeds		7	15	7	7	9
Number of perennial weeds		1	2	2	2	2
Aboveground fresh weight of weeds (g m^{-2})		40.9	267.8	242.2	404.6	536.6
Aboveground dry weight of weeds (g m^{-2})		11.0	56.3	43.5	97.4	130.2

Table 4. Average weediness in period of investigation

	Cropping system (B)				
Weed characteristics	1	2	3	4	5
Total number of weed species	7.1[a]	13.1[c]	7.8[a]	7.9[a]	9.6[b]
Total number of plants per species	13.1[a]	22.3[b]	21.6[b]	14.6[a]	20.8[b]
Aboveground fresh weight of weeds (g m^{-2})	48.5[a]	285.4[b]	286.4[b]	394.4[b]	564.3[c]
Aboveground dry weight of weeds (g m^{-2})	13.5[a]	58.7[b]	47.2[b]	92.9[c]	137.5[d]

Values of means followed by the same letter are not significant at the level of 0.05;

Observing the two control variants (dead organic mulch and conventional treatment) in both years, based on data in Table 4, we see a number of weed species, weed plants and especially higher values in the fresh weight of weeds in the conventional system in relation to the dead organic mulch variant. Thus, the mulch system in sweet maize is more favourable in terms of reducing the number of species, number of individuals, especially in terms of fresh weight of weeds. Increasing the biodiversity and number of crops in year per unit area, as in cover cropping, leads to a reduction in the biomass of weeds.

Table 5. Statistical analysis of observed weed parameters in sweet maize (lsd)

lsd	Total number of weed species			Total number of plants per species			Aboveground fresh weight of weeds			Aboveground dry weight of weeds		
	A^{**}	B^{**}	AB^{ns}	A^{ns}	B^{*}	AB^{ns}	A^{ns}	B^{**}	AB^{ns}	A^{ns}	B^{**}	AB^{ns}
0.05	1.3	2.1	2.9	3.8	6.0	8.5	73.6	116.4	164.7	16.7	26.5	37.4
0.01	1.8	2.8	4.0	5.2	8.2	11.6	100.9	159.7	225.8	22.9	36.3	51.3

$p<0.01$ very significant (**); $p<0.05$ significant (*); $p>0.05$ no significant (^{ns});
A-year of investigation, B-cropping system.

Conclusion

Based on results, obtained effects of the cover crops on weed infestation of sweet maize grown on chernozem under rain fed conditions, the following can be concluded:

The weed community was composed of a relatively small number of weed species and number of plants per species–11.6 and 19.5 (2012) and 6.6 and 17.5 (2013). The dominant weed species in the summer aspect of sweet maize were *Sorghum halepense* L. Pers., *Datura stramonium* L., *Chenopodium album* L., *Amaranthus hybridum* L., and *Solanum nigrum* L. and significant places in weed synusia were taken up by *Amaranthus retroflexus* L., *Amaranthus albus* L., *Convolvulus arvensis* L., and *Hibiscus trionum* L.

Differences in the number of weed plants per species, as well as in fresh and air dried weed biomass obtained among observed investigation years were not statistically significant.

The cover crop with common vetch expressed greater efficiency in weed control (number of species, number of plants per species and weed biomass) in comparison to different cropping systems. All differences in the investigations parameter of weeds in comparison with conventional and mulch cropping system was very statistically significant.

The system of cropping cover crops under rain fed conditions before sweet maize expressed a significant advantage in weed control particularly in troublesome perennial species in relation to traditional cropping system of sweet maize.

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EFFICIENCY OF BIOLOGICAL AGENTS IN CONTROLLING OF SEEDS AND ROOTS DISEASES OF EGGPLANT

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Abstract

The effectiveness of alcoholic extract of Oak gall and Neem seeds on the integrity of eggplant seeds was examined. Five different seed samples were collected from the northern provinces of Iraq and used in this study. The result of our examination showed presence of various fungi including *Aspergillus, Aurobasedium, Botrytis, Cladosporium, Fusarium, Phoma* and *Rhizoctonia* in the examined seeds and roots samples. Isolation rate of fungi ranged from 1% to 17%. Fungi that were isolated from studied roots included *Alternaria*, *Botrytis, Chaetomium, Cladosporiu, Drechslera, Fusarium, Geotrichum, Macrophomina, Phytophthora, Pythium, Rhizoctonia, Sclerotium* and *Stemphyllium*. Alcoholic extract of Oak gall showed inhibitory effect of 8% and 10% against *F. oxysporum* and *F. semitectum* respectively. Whereas, Alcoholic extract of Neem seeds showed inhibitory effect of 64% and 61.77% against *R. solani* and *F. oxysporum*, respectively. The soil-borne fungus *Trichoderma harzianum* showed an antagonistic activity, and it had antifungal effect against several pathogenic fungi, especially *F. solani*, with antibiosis level of 1.66. *Bacillus subtillis* also showed high inhibitory activity of 100% against *F. semitectum* and 92.66% against *F. solani*.

Key words: *Eggplant, Biological Control, Fungi, Neem, Oak gall*.

Introduction

Biological control is an effective method against diseases transmitted by seeds. It is well known that, this method positively affect the activity of most microorganisms, and it improves soil conditions and sustainability of agriculture (Papavizas and Lewis, 1989). Biological control method is also proven to cause no harm to environment, and it reduces human health risks. It is believed that, Eggplant, *Solanum melongena L*., is originated in China and India. It grows as a wild plant (Chen and Li, 1996), and it usually needs a warm climate for growth. Generally, eggplant is economically important vegetable crop, and it ranks third after potatoes and tomatoes in terms of world production. The plant is cultivated in tropic and subtropics region of Iraq, and the total area of planting is 21,665 ha and production is 24,321.84 kg /ha (anonymous, 2001) .The plant is grown seasonally in open fields in spring, to produce fruit during summer months and autumn. Due to its importance and increased demand in winter and early spring, cultivation of this crop in protected facilities began in 1980s in Iraq to enhance and extend its production capacity (Bozorgi, 2012). The available information on the most producing countries of eggplant in descending order are: China (18 million tons), India (8.4 million tons), Egypt (1.2 million tons), Turkey (813 thousand tons), Indonesia (389 thousand tons), Iraq (380 thousand tons), Japan (371 thousand tons), Italy (321 thousand tons), and Sudan (230 thousand tons) (FAO, 2008). However, eggplant is susceptible to many diseases, particularly fungal infections. Seeds play a vital role in transferring and spreading of these diseases. The damaging effect of diseases to crops, and massive economic loss as a result was indicated in a previous study, where the study stated a substantial economic loss (up to100%) which was related to vascular wilt diseases (Alabouvette *et al*., 1996).

This study aimed to investigate seeds and roots diseases of eggplants that were collected from three Northern provinces in Iraq, and to determine the efficiency of two different biological methods in controlling of such diseases.

Material and methods

Isolation and diagnosis of fungi associated with seeds:

Five samples of eggplant seed lots were collected from several local companies and farmers in three provinces Akrae in Dohuk, Erbil, and Koya in Sulaimani (Iraq).

The samples were kept in polyethylene bags in the refrigerator at 5°C until use. Fungi were isolated from seeds using agar plate method that was described by the International Seed Testing Association (ISTA, 1976). A 200 seeds were surface-sterilized by immersion in 1% sodium hypochlorite (NaOCL) for three minutes. Distilled water was used to wash the seeds thoroughly, and then seeds were dried between two layers of the Whattman filter paper. The treated seeds were distributed in separate dishes. Each ten seeds were placed in a dish containing 15 ml of PDA with streptomycin sulfate (50 mg /L).Plates were kept at 25°C for 12 h under light and 12 h under dark regime for one week. After the incubation period, plates were examined for the presence of fungal growth, and the detected fungal colonies were identified according to (Booth, 1971; Toussaun and Nelson, 1976; Pitt and Hocking, 1997; Leslie and Summerell, 2006). The isolated colonies were purified by using single spore or hyphal tip technique, and preserved on PDA slant at 5°C until used.

Isolation and diagnosis of fungi associated with roots:

Several field-infected plants were transferred to Plant Protection laboratory at the Faculty of Agriculture, University of Salahaldeen. Isolation of fungi from the infected plants was performed according to the method described by Agnihorti, (1971). Following a week of incubation, the colonies were examined and prepared as mentioned in the preceding paragraph and preserved on PDA slant tubes at 5°C.

Pathogenicity of isolated fungi:

Pathogenicity of the isolated fungi from the infected seeds and seedlings was tested. The PDA medium was used for cultivation of fungi, and cultivated plates incubated at 25°C°+2. The grown fungi colonies were used in inoculation of sandy loamy soil. For this experiment 2 kg sandy loamy soil was used. The soil then inoculated with the fungi colonies grown on one half of the PDA plate. The added fungi growth mixed thoroughly with the surface layer of the soil (Saydam *et al.*, 1973). Following mixing, the soil distributed in 1% formalin sterilized 20 cm-diameter pots. The experimental pots were placed in the canopy of wood for three days. Eggplant seeds that had been obtained from Erbil were used for the experiment at rate of 10 seeds per pot. Prior to spreading of seeds on the pots, seeds were sterilized with 1% sodium hypochlorite solution for three minutes. After sterilization, the seeds washed with sterilized distilled water, and spread on contaminated soil.

Biological control: a) Plant extracts:

Two types of forest tree extracts were tested to assess their effectiveness against pathogenic fungi. Oak gall was obtained from Akrae, and Neem seeds were obtained from Sami Abdul Rahman Park in Erbil. Both Oak gall and Neem seeds washed with distilled water, and then placed on a filter paper at room temperature to dry. Each dried plant was kept in a labeled polyethylene bag until use.

A twenty gram of each oak gall powder and Neem seed powder was separately added to 200 ml of 95% ethanol in 400 ml flasks. The mixtures left in shaking condition in water bath (Karlsruhe Company-Germany) for 1 hour, and then kept in refrigerator at 4ºC for 24 hours. The extract was filtered through two layers of gauze, filtrate placed in a petri dish and left to dry at room temperature. The alcoholic plant extracts then collected in glass bottles and stored at -20ºC in deep freezer until use (Alnoman, 1998).

One gram amount of alcoholic extract was added to 5 ml of dimethyl sulfoxide (DMSO) (BioSolve BV, Netherlands). The mixture then filtered using Millipore filters (0.2 micrometer). A 10 mg /ml of the above substance were added to 500 ml sterile PDA medium, and the medium poured into sterile glass petri dishes. The same procedure was followed in preparation the other concentrations (8, 6, 4, and 2 mg /ml). Freshly prepared disc (0.5 cm diameter) of pathogenic fungal colonies was placed over the prepared plates. Each treatment was repeated three times, control containing medium without alcoholic plant extract was also included in the experiments (Riso *et al*, 1987).

b) With fungi and bacteria: Antagonism activity test carried out by using the bio-agent *T. harzianum* against pathogenic fungi that were grown on PDA plates. The plate was divided into two parts, a disk (0.5 cm diam.) containing colonies of *T. harzianum* was placed on the first half of the medium. While another disk (0.5 cm diam.) containing colonies of pathogenic fungi was placed on the second half of the medium. The distance between both microorganisms was almost 4 cm. The experiment repeated three times. Control was also included in the experiment, where, *T. harzianum* was used to inoculate a centre of a medium plate, and a disk containing pathogenic fungi was used to inoculate a different plate. The results obtained after a week of incubation at 25ºC, and evaluated according to Bell *et al.*, (1982).

Antagonism test of *Bacillus subtillis* against fungal pathogens:

Each PDA plate was inoculated with 0.5ml of *B. subtillis* at rate of 2.5×10^8 cfu/ml. The bacterial culture was grown in a nutrient broth at 37 ºC for 24 hours, and 0.1ml of a fresh culture aseptically spread on the surface of the plate. Inoculated disks with fungal cultures were placed at the center of PDA plates, and plates were incubated at 25 ºC. Experiment for each fungi culture was repeated three times. Results of grown fungal colonies on plates were recorded and the ratios of Qatrin perpendicular and the percentage of inhibition were calculated. Generated data was statistically analyzed using the Statistical Analysis System-SAS (Anon, 2005), and validated by Duncan’s multiple range test.

Results and discussion

Isolation and diagnosing of fungi associated with seeds

The testing result of seeds integrity of five eggplant samples that were obtained from Erbil, Dohuk, and Sulaimani is shown in (Table 1).

Table 1. Fungi isolated from the seeds of eggplants

Governorates	Fungi	%Isolation	% Frequency
Erbil	*Aspergillus spp*	3	27.3
	Phoma sp	1	9.1
	Rhizoctonia solani	7	63.6
Sulaimani	*Aspergillus spp.*	5	100
Dohuk	*Aspergillus spp*	4	57.1
	Botrytis cinnerea	3	42.9
Akrah	*Aspergillus spp*	2	20
	Aurobasidium pullulans	1	10
	Cladosporium cladosporioides	1	10
	Fusarium oxysporum	1	10
	Fusarium solani	1	10
	Rhizoctonia solani	4	40
Koya	*Aspergillus spp.*	3	100

The table shows that *R. solani* was found in 7% of examined seeds, with the highest rate (63.6%) of frequency which was recorded in seeds collected from Arbil. This finding is agreed with the findings of (Ismail, 2010), who indicated isolation of fungi from eggplant

seeds. In addition to *R. solani*, *Aspergillus* sp. was also found, and the later fungus presence was recorded in all examined samples at a range between 2-5%. The highest debut (100%) was found in seeds obtained from Sulaimani, and the lowest (20%) was found in seeds obtained from Akrae. A similar result was showed in a previous study by (Al-Kassim and Monawar, 2000), who also stated isolation of these fungi from seeds of eggplant. Moreover on fungal isolation from eggplant seeds, *F. oxysporum* was also isolated from the seeds obtained from Akrae at rate of 10%. A similar result regarding isolation the latter fungi from seeds were also reported by Ismail (2010). However, it is noteworthy to mention that *F. oxysporum* can cause a significant loss in production through causing diseases such as wilt disease. Symptoms of the disease usually occur on mature plants and unilateral gestures. Yellowing may appear on one side of infected plant then extend to all parts followed by wilt (Siva *et al*., 2008).

In a recent study, researchers were able to isolate both *F. oxysporum and F. solani* from seed embryos of eggplant (Habib et al., 2007). The occurrence of *F. solani* in soil is common, and contaminated seeds are the main route of transmission. This fungi can cause root rot diseases and stem ulceration in various plants (Gordon, 1960), and it considered one of the most dangerous pathogenic fungi that can cause wilting (Chakraborty and Chatterjee, 2008).

Isolation of fungi from roots of eggplant:

Our data in Table 2 shows presence of *Fusarium* sp. in all samples that were collected from all studied sites in the regions of Erbil with repetition rate of 66.66%.

Table 2. Fungi isolated from the roots of eggplants in areas of the province of Erbil

Fungi	Khabat		Ainkawa		Shmamk		Total	
	% Iso	% Freq	% Iso	% Freq.	% Iso.	% Freq.	% Iso	% Freq.
Alternaria alternata	7	8.33	1	1.47	1	2.38	9	4.6
Botrytis cinnerea	5	5.95	-	-	-	-	5	2.7
Chaetomium herbarium	-	-	-	-	1	2.38	1	0.5
Cladosporium cladosporioides	-	-	-	-	1	2.38	1	0.5
Drechslera spp.	6	7.14	2	2.94	-	-	8	4.1
Fusarium compactum	1	1.19	-	-	1	2.38	2	1.0
Fusarium miscanthi	1	1.19	-	-	-	-	1	0.5
F. semetictium	1	1.19	1	1.47	-	-	2	1.0
F. solani	3	3.57	1	1.47	2	4.76	6	3.1
Fusarium spp.	39	46.4	33	48.52	28	66.66	100	51.7
Geotrichum candida	-	-	1	1.47	-	-	1	0.5
Macrophomina phasiolina	-	-	-	-	1	2.38	1	0.5
Phytophthora spp.	-	-	1	1.47	2	2.38	3	1.5
Pythium spp.	3	3.57	3	4.41	1	2.38	7	3.6
Rhizoctonia solani	15	17.9	15	22.05	4	9.52	34	17.6
Sclerotium spp.	3	3.57	4	5.88	-	-	7	3.6
Stemphyllium herbarum	-	8.33	6	8.82	-	-	6	3.1
Total of Posative samples	84		68		68		194	
Total of negative samples	16		32		32		106	

- Non-appearance of fungi. Isolation, ** Freq. = Frequency. =Iso. = *

However, information of the conducted field survey demonstrated isolation of *F. solani* from several studied sites especially in Shmamk, Khabat, and Ainkawa at rate of 4.76%, 3.57%, and 1.47% respectively. In addition to what we have explained earlier, as these fungi are commonly found in soil and can be transmitted through seeds, they can also penetrate plants through wounds on lateral roots or through the exposure to undesired conditions. Because its parasitic nature, it can cause root-rot diseases to many plants (Gordon, 1960), and

subsequently the infected plants develop symptoms such as taproot and lateral roots browning. The later description of fungal root infection was also indicated in another study were the researchers stated that, the infection that had been occurred in the root reflected first on the aerial parts then yellowing and drought without discoloration of the vascular vessel (Ahmad and Al-Moussalli, 1987). In addition to *Fusarium* sp., *R. solani* was also isolated from all of the surveyed sites with the highest incidence found in Ainkawa 22.05%, Khabat 17.85%,and Shmamk9.52%. However, its occurrence may due to the contamination of soils in these sites with this fungus, which is considered as one of the most causal agent of rot and seedling death to its wide range host that may exceed 230 plant species. Especially when we conclude that, *R. solani* can remain in the case of vegetative growth in the wet soil for the rest of the year (Parameter,1970).However, during infection process; the fungi affect seeds and seedlings in the soil before and after emergence.At the final stage, the disease attacks the roots and aerial parts of affected pots, fruits, leaves and stems (Anne et.al, 2002).

Pathogenicity of fungi isolated from eggplant:

Seed rot and seedling death of eggplant.

Results regarding seed rot and seedling death of eggplant collected from the region of Erbil are shown in Table 3.

Table 3. Effect of some fungi isolated from seeds and roots of eggplant rot in the Pre-emergence damping-off and seedling death Post-emergence damping-off of seeds obtained from the province of Erbil

Fungi	% Pre- emergence damping–off	%Post- emergence damping–off	% Survival plants
Control	13.3 b	3.3 de	83.3 a
Alternaria alternata	26.7 ab	6.7 cde	66.7 ab
Drechslera spp.	33.3 ab	10.0 bcd	56.7 abcd
Fusarium compactum	43.3 ab	10.0 bcd	46.7 abcd
F. miscanthi	36.7 ab	0.0 e	63.3 abc
F. oxysporum	63.3 a	10.0 bcd	26.7 cd
F. semetictium	53.33 ab	16.66 b	30.0 bcd
F. solani	63.33 a	13.33 bc	23.33 d
Rhizoctonia solani	43.33 ab	36.33 a	20.33 d

Numbers that bear similar letters do not differ significantly at 0.05 probability level using Duncan multiple range test (vertically) .* Each number represents the average of three replicates

F. solani and *F. oxysporum* were the most virulent fungi isolated from seeds at rate of (63.3%) without showing any significant differences between the species of fungi except control treatment. Our result also shows contribution of *F. semitectum*, *F. copactum* and *R. solani* in causing seed rot that reached 53.3% and 43.3 % respectively. However, *A. alternata* caused pre-emergence damping-off at less extent 26.7%. And, *R. solani*, *F. miscanthi*, and *F. solani* caused the post emergence damping-off at rate of 36.3%, 16.7%, and 13.3% respectively. Meanwhile, *F. miscanthi* did not cause death of seedlings and the surviving percentage of the infected plants with *F. miscanth* was 63.3%, which indicates a weak pathogenicity of these fungi. While, surviving percentage the infected plants with *R. solani* reached 20.3%, which refers to high pathogenicity.

Biological Control

Effect of alcoholic extract of Oak galls on fungal growth:

The effect of alcoholic extract of Oak gall in inhibition growth of pathogenic fungi is shown in Table 4.

Table 4. Effect of alcohol extract of oak galls in the growth of *Fusarium* species pathogenic to eggplants

Pathogenic Fungi	Control (Cm)	Concentration				
		2%	%4	6%	8%	10%
		%Inhibition				
F. compactum	7.3 a *	28.4 a	46.7 a	52.3 a	55.7 a	61.4 a
F. miscanthi	6.2 b	35.1 b	50.0 b	60.9 b	62.2 b	64.9 b
F. oxysporum	6.8 a b	76.6 c	80.3 c	85.3c	100.0 c	100.0 d
F. semitectum	6.7 ab	62.5 c	80.0 c	81.2 c	100.0 c	100.0 d
F. solani	6.0 b	32.0 b	37.5 ab	59.8 b	65.3 b	73.7 c

Numbers that bear similar letters do not differ significantly at 0.05 probability level using Duncan multiple range test (vertically). * Each number represents the average of three replicates.

The inhibition rate of the extract at 10% concentration against *F. compactum* found to be 61.4% with no significant differences between the treatments and the control plants. On the other hand, testing the impact of alcoholic extract of Oak gall on *F. miscanthi* showed the highest inhibition rate of 10% of the extract was 64.9%, and the lowest inhibition rate of 2% of the extract was 35.1%. When the influence of Oak gall extract was tested against *F. oxysporum*, a significant inhibiting action (100%) of the extract on the fungus was obtained. Although, there were no significant differences among the concentrations used, but a difference was found between control plates and the extract at 2% concentration, where the lowest inhibition rate 76.6% was observed. Moreover, the 10% alcoholic plant extract also had a significant inhibition action (100%) on the growth of *F. semitectum*. Meanwhile, the 2% extract showed the lowest inhibition rate of 62.5% with no significant differences among tested concentrations. The differences were only found with the control. However, *F. solani* was found to be the most susceptible to the plant extract treatment. Similar conclusion was also stated by Yamunarani, *et.al*.,(2005),who indicated inhibition of fungi growth by protein extracted from Oak. Söhreto *et al*., (2007) also mentioned the impact of alcoholic extract of Oak galls on mycelium growth of these pathogenic fungi.

Effect of alcoholic extract of Neem seeds on fungal growth:

The effect of alcoholic extract of Neem seeds in inhibiting growth of pathogenic fungi is shown in Table 5.

Table 5. Effect of alcoholic extract of neem seeds in inhibition the growth of *Fusarium* species pathogenic to eggplants.

Pathogenic Fungi	Control cm	Concentration				
		2%	%4	6%	8%	10%
		% Inhibition				
F. compactum	7.33 a*	14.7 a	27.28 a	30.7 a	46.7 a	53.5 a
F. miscanthi	6.16 b	17.5ab	17.53 a	22.9 ab	44.6 a	58.1 a
F. oxysporum	6.75a b	44.4 b	48.14 b	48.1 b	55.6 a	61.8 a
F. solani	7.33 a*	9.8 ab	27.28 a	26.5 ab	50.0 a	60.1 a

Numbers that bear similar letters do not differ significantly at 0.05 probability level using Duncan multiple range test (vertically).* Each number represents the average of three replicates.

The inhibition level of alcoholic extract of Neem seeds on *F. solani* was found to be 64.0% with no significant differences between treatments. A similar finding was also indicated by other researchers (Carpinella *et al*., 2003). Increasingly, *F. oxysporum* was also negatively impacted by this seed extract; the inhibition level was 61.8%. The highest inhibition level was observed at concentration of 8 and 10%**.** This particular result was in contrast with the result obtained by Hassanein *et al*., (2008) who found the inhibition rate of Neem plant extract at

20% concentration on the growth of *F. oxysporum* and *F.solani* to be 7%. But the inhibition rate of the same concentration on the growth of *F. semitectum* was 60.1%. The impact of 8 and 10 % extract concentration was found to be significantly different from the rest of the treatments. On the impact of the extract on the growth of *F. miscanthi*, the extract's inhibition rate was 58.1% with no significant differences between all treatments. Effectiveness of the alcoholic extract of Neem seeds may be attributed to its content of paraisine, which is one of the alkaline substances that inhibit fungi growth. It may also due to its combination with the dynamic biological processes, including the precipitation of the amino acid in the proteins synthesis (AL-Rawi and Chakravaraty, 1964). Another reason it could be containing materials effective against fungi including Vanillin and 4-hydroxy-3- methoxycinnam aldehyde and Pinoresinol (Carpinella *et al*., 2003).

Biological control using bacteria and fungi:

Efficiency of *Trichoderma harzianum* against pathogenic fungi.

The result presented in Table 6 indicates the high antagonism ability of *T. harzianum* against pathogenic fungi that infected eggplants.

Table 6. The efficiency of biocontrol *Trichoderma harzianum* against fungal pathogens of eggplants

Fungi	Degree of Antagonism *
Alternaria alternata	**2.3
Drechslera spp.	**2.00**
Fusarium compactum	**2.2**
Fusarium miscanthi	**2.0**
Fusarium oxysporum	**2.0**
Fusarium semetictium	**2.0**
Fusarium solani	**1.7**
Rhizoctonia solani	**2.3**

* World peace quintet Bell *et.al*., (1982). ** Each number represents the average of three replicates.

The antagonism level against *F. solani* found to be 1.7 degree, followed by *Drechslera* spp. Our results were in line with the results observed by other researchers, (Küçük and Merih, 2003) emphasized the ability of different bio- control such as *T. harziurum* in inhibiting growth of *Drechslera sorokiniana, F. oxysporum* and *R. solani*. In regard to *F. miscanthi, F. oxysporum* and *F. semitectum* the degree of antagonism level was found to be 2.0, while the degrees of antagonism against *A. alternate , F. compactum* and *R. solani*, were 2.3, 2.2, and 2.3 respectively. These results are consistent with the results reported by Wahgunde and Patil, (2009) in relation to the antagonism ability of the fungi *T. viride* and *T. harziurum* in inhibiting the growth of *A. alternate.* The antagonism ability of bio-control *T. harzianum* can be attributed to the production of B-1-3-glucanase enzyme, which weakens the pathogenic fungi and releasing cellulose enzymes in small quantities. However, this may assist in the penetration process of the bio-controls to pathogenic fungi cells and production of antibiotic, which degrades fungal thread (Benhamon and Chet, 1993).

Proficiency test of bacterial antagonism activity against pathogenic fungi:

The bacterial antagonism activity against pathogenic fungi was tested by using *B. subtillus*. The results show that the inhibitory affect of *B. subtillus* was highest (100%) on *F. miscanthi.* On the other hand, it showed no effect on the growth of *Drechslera sp*. However, in a previous study of Korston and de Jager (1995) the authors found a remarkable antagonism activity of this bacterium on spores growth and mycelia of *Drechslera setariae*. While, the inhibition rate reached up to 92.7% against *F. solani*. In another previous study,

Kim, *et.al*., (1994) have also reported a potential antagonism activity of same bacteria on the growth of *F. solani*. Where the authors concluded the following, treated eggplant seedlings with *B. subtillus* prior to planting in contaminated soil reduced seedling infection rate from 68 % to 76 % by *F. solani*, and 90.8% by *F. oxysporum* within 25 to 30 days after treatment. However, this antagonism activity may be due to the ability of these bacteria in secretion group of antibiotics which include Difficidin, Prydifficidin, Bactracin, and Bacillomyin. It may also attribute to their ability in producing volatile organic compounds. Mahammad and Amusa, (2003) indicated a clear inhibitory impact (43-93%) of a multiple volatile organics that were produced by this bacteria (alkyls alcohols, esters, ketones and acid, oxime, and heterocyclic amine phenols) on the growth of fungal mycelium.

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ASSESSMENT OF BISEXUAL P. ATLANTICA GENOTYPES AS POLLINATORS FOR P. VERA CULTIVARS AND THE GENETIC SIMILARITY AMONG THEIR F1 PROGENIES USING SSR MARKERS

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Abstract

The research was conducted in the Agricultural Scientific Research Centre in Sweida-GCSAR (Syria) on two *P. vera* female cultivars (Ashoury and Batoury) using 3 bisexual *P. atlantica* genotypes (PA12, PA35 and PA37) and open pollination as control. Pollen viability was evaluated using IKI (Iodine + Potassium Iodide) and TTC (2,3,5-Triphenly Tetrazolium Chloride) dyes. *In vitro*-germination using hanging drop method was used to evaluate fertility before cross-pollination. In all tests, the bisexual *P.atlantica* genotypes (PA37, PA35) gave high pollen viability and fertility percentages. The ability of these pollinators was evaluated according to the most important economic criteria which used to evaluate the behaviour of *P.vera* verities as set percentages, nut's diameter, spherecity, and split percentage. Genetic relationship among F1 hybrids and their parents was achieved using 20 specific SSRs primer pairs, 16 of them were able to amplify PCR products and revealed 44 alleles, of which 40 were polymorphic (90.91%). Genetic similarity ranged from 0.24 to 0.96. The UPGMA (Unweighted Pair Group Method using Arithmetic Averages) cluster based on Jaccard's coefficient grouped the genotypes into two main clusters. The number of alleles ranged from 1 to 9, with a high level of expected heterozygosity (He) 0.496, and 0.25 of observed heterozygosity (Ho). Marker Index (MI) was 18.94. These results indicated that SSR (Simple Sequence Repeat) marker is an informative technique which played an important role as genetic marker for lineage studies.

Keywords: *Bisexual genotypes, crossing, pollen fertility and viability, SSR marker.*

Introduction

The genus *Pistacia* belongs to *Anacardiaceae* family and comprises eleven species, some of which are of high economic and cultural importance in Mediterranean and Asian countries (Isfendiyaroglu, 2007). All *Pistacia* species are dioecious and their flowers are unisexual except of some individual specimens of *P.atlantica* and *P.terebinthus* species (Kafkas, 2002; Gercheva*et al*, 2008). Two new specific bisexual genotypes of *P.atlantica* were identified in Sweida province; the first genotype showed that all the flowers in the raceme are bisexual with only 1-2% of female flowers, whereas the other genotype has four patterns of racemes (Alhajjar *et al*, 2011). These exceptional transsexual genotypes are of importance in relation with pistachio breeding programs. However, Pistachio breeding program faces a lot of complicated obstacles, since it is dioecious species and therefore the potential of male parents is unknown (Romero *et al*., 2001). Morphological criteria remain unsatisfied, from the fact that one cultivar may express its behavior in different ways according to the ecological conditions, in this prospect; molecular markers could facilitate breeding and allow early seedlings selection, saving time and economic resources (Vendramin *et al.*, 2009). Turkeli and Kafkas (2013) assessed the first linkage map in pistachio using an interspecific cross between *Pistacia vera* L. (siirt cultivar) and bisexual *Pistacia atlantica* Desf. (Pa-18 genotype), using ISSR, SRAP and AFLP markers. SSR markers are multi-allelic, co-dominant genetic markers with a very high repeatability, and so are particularly suitable for

phylogenetic studies because of their high polymorphism and abundance (Gupta *et al*., 1996). In the current work, breeding program was assessed using three bisexual *P. atlantica* genotypes as donators and two Syrian *P. vera* female cultivars (Ashouri and Batouri) as receptors, to evaluate viability and fertility of the pollen grains and thus to demonstrate their potential efficacy, and to determine the genetic relatedness of F1 progenies with their parents (*P. vera* × bisexual *P. atlantica*) using 20 SSR markers.

Material and Methods

This research was conducted at the Agricultural Scientific Research Center in Sweida Province- GCSAR during 2012-2014, and in pistachio fields localized in the rural area.

Plant material

Female cultivars:

Ashouri: It is the Syrian world wild cultivar (red Aleppo), consists about 75% in the mentioned area.

Batouri: It consists about 20% of the cultivated cultivars in the mentioned area.

Bisexual *P. atlantica* genotypes:

PA12: It contains two types of sexual racemes; 50% female and 50% male racemes. Pollen grains are coved with a sticky substance inside the anthers.

PA35: It has bisexual flowers with an anther located over the oval near the stigmata.

PA37: It has bisexual flowers with an anther located on a short base at the bottom of the oval. Also, the flower sometimes consists of two anthers with a stigmata between them. And some branches contain female racemes.

Viability and fertility tests:

Iodine Potassium Iodide (IKI) and 2,3,5- Triphnyle Tetrazolium Chloride (TTC) were used to estimate the viability of pollen grains according to (Dalkilic and Dogru, 2011).

In vitro-germination – hanging drop method was used to estimate pollen grains fertility using sucrose nutritional medium (Acar *et al*., 2010).

Cross pollination:

Pollen grains of the selected genotypes of the bisexual *P.atlantica* Desf. (PA12, PA35, PA37) were used, as well as open pollination as control. It was applied when the stigma of the two cultivars had become receptive. Nuts parameters as nut dimension and sephercity, set nut percentages, and split nut percentages were estimated.

DNA Extraction

It was achieved using CTAB protocol according to (Porebski *et al*, 1997). DNA quantity and quality were estimated using 1% agarose gel electrophoresis by comparing band intensity with 1 Kb ladder of known concentrations.

PCR amplification

Extracted DNA was PCR-amplified using 20 SSR primer pairs according to (Ahmad *et al.*, 2003; Vendramin*et al.*, 2010).

Data analysis

For cytogenetic studies, randomly simple replicates design ($P \leq 0.01$) was used. For cross pollination; one way anova ($P \leq 0.05$) was used. Genetic similarity (GS) was calculated from the alleles across the 20 SSR loci using Jaccard’s similarity coefficients (Jaccard, 1908). A dendrogram was constructed with the unweighted pair group method using arithmetic averages UPGMA (Sneath and Sokal, 1973). Observed heterozygosity (Ho) Wunch and Hormaza, (2007), expected heterozygosity (He) Lorenzo *et al*. (2007), and Marker Index (MI)

according to Powell *et al*. (1996) were also determined to estimate the efficiency of SSR technique. The software used through this study was Gen Stat for cytogenetic studies and nuts parameters, and Microsoft EXCEL, SPSS17, and Past for genetic analysis.

Results and discussion

Pollen grain viability

The results showed that the IKI test, revealed an average of viability percentage ranged from 79.74% to 97.11% (Table, 1). The highest percentage of viable pollen grains was in PA37 genotype (97.11%), followed by the genotype PA35 (96.77%). In TTC test, the viability percentage in all genotypes was nearly higher compared with the IKI test. So, the highest value was 98.17% in the genotype PA37 and 97.06% in PA35 genotype (Table, 1). The present results are in agreement with the previous investigations, Sfendiyaro *et al* .(2006) reported that pollen viability in TTC test ranged between 85.7-98.85% in different mail types.

Pollen grain fertility

Fertility is one of the most important criteria when estimating pollen efficiency in breeding programs. Results in Table (1) indicated to the accordance between viability and the fertility. Pollen germination was estimated as a percentage when the germination tube reached half diameter of the pollen grains. The highest fertility percentage was 88.61% in PA37 genotype, whereas the lowest percentage was 79.22% in PA12 genotype.

Table1. Bisexual *P.atlantica* pollen viability estimated by using IKI and TTC tests and fertility%.

genotype	IKI test			TTC test		Fertility%
	Semi viable	viable	unviable	semi viable	viable	
PA37	1.83	98.17 ^{A}	1.06	1.83	97.11 ^{A}	88.61^{A}
PA35	2.94	97.06 ^{A}	2.01	1.22	96.77 ^{A}	85.49 ^{A}
PA12	5.39	95.36 ^{B}	3.8	2.17	94.03 ^{B}	79.22^{B}
LSD 1%		1.49			1.66	4.57

Nuts set percentage

Table (2) showed that ‘Ashoury’ has got the highest nuts set percentage than ‘Batoury’ in all applied pollinizers. The highest nuts set percentage in ‘Ashoury’ significantly obtained by using PA37 bisexual *P.atlantica* pollen grains (62.60%) and PA 35 (59.90%) in comparison with the other pollinizer and the control. The best pollinizer for ‘Batoury’ was PA35genotype (21.46%) followed by open pollination 21.21%.The present results were disagreement with the previous investigations (Isfendiyaroglu *et al*., 2001; Kardoush *et al*., 2009), and in agreement with (Ozeker *et al*., 2005).

Nuts dimensions and sphericity

Nuts dimension was differed with respect to source of pollen. Within ‘Batoury’ replicates using different pollinizers, the pollinizers PA37 and PA35 significantly gave higher nuts dimensions (2.46 cm and 2.35cm) in comparison with the PA12 and the control (open pollination) (Table,2). Within ‘Ashoury’ replicates, the bisexual *P.atlantica* genotypes (PA37, PA35) were able to increase the nut size 1.92 and 1.90 cm with significant differences in comparison with the control (1.61 cm). Ak (1999) produced nuts of 0.87 cm by using *P.atlantica* pollinizer. The sphericity percentage ranged between 77.47 - 88.81% in ‘Batoury’, and between 64.11 -72.03% In Ashoury. Kardoush *et al*. (2009) reported that the sphericity of ‘Ashoury’ cultivar was 73.9%. And in ‘Batoury’ the nut sphericity was 82.8% when *P.atlantica* pollen grains were used and 68.8% with open pollination. Fares *et al.* (2009) mentioned that the dimension of pistachio nuts ranged from1.63 cm to 2.04 cm.

Split shell percentage

Various split shell values were obtained in both cultivars according to the source of pollinizer. In Ashoury, the genotypes PA35 and PA37 significantly raised the percentage of split nuts (93.75- 90.99%) in comparison with open pollination (83.33%). In ‘Batoury’, the genotypePA37 significantly revealed the highest percentage of split nut (35%), the least split nut percentage (12.00 %)was obtained by using *P.atlantica* PA12 genotype (Table 2).

Table 2. set percentage, loss percentage, dimension/cm, shephrecity%, and split %

Cultivar/ polliniser	**Set %**	**Dim. cm**	**sphericity%**	**split%**
Bat/PA35	21.46 cd	2.46 a	88.81 a	20.00 f
Bat/PA37	20.10 de	2.35 a	84.78 b	35.00 e
Bat/Contr	21.21 cd	2.07 b	78.79 c	19.12 f
Bat/PA12	12.83 e	2.04 b	77.47 c	12.00 g
Ash/PA37	62.60 a	1.92 bc	72.03 d	90.00 b
Ash/PA35	59.70 a	1.90 bc	69.84 d	93.75 a
Ash/PA12	28.45 bc	1.78 cd	65.33 e	69.23 d
Ash/Contr	34.44 b	1.61 d	64.11 e	83.33 c
LSD 5%	**7.89**	**0.19**	**2.73**	**2.64**

Polymorphism detected by SSR marker

Twenty SSR primer pairs were applied, 16 of them were able to detect polymorphism and revealed 44 alleles across all genotypes, 40 alleles were polymorphic (90.91%). These results were in agreement with Arabnezhad *et al.* (2011), they used 18 SSR primer pairs developed from *P. khinjuk* genome. All primer pairs produced 1-9 putative alleles each with an average 2.75 alleles per locus, and the highest number of putative alleles was revealed in the progeny HB3 (FB×PA35), Table (3). Baghizadeh *et al.* (2010) reported that four SSR primers produced 11 alleles with an average value of 2.75 alleles. However, the primer pairs (Ptms-11, Ptms-33, Ptms-40, and Ptms-42) revealed monomorphic alleles, whereas the other primer pairs revealed polymorphic alleles. The progeny HB3 (FB×PA35) was distinguished by 6 alleles using EPVM021 primer pairs, 3 of them were unique alleles (491, 338 and 307bp, respectively) as shown in (Table, 3). Heterozygosity was detected by EPVM016, EPVF019 and Ptms-7 primer pairs. Allele size ranged between 76 bp in Ptms-11 primer to 690bp in EPVM021 primer.

Table 3: Total number of alleles produced by 14 SSRs primer pairs, number of polymorphic alleles, polymorphism percentage and allele size (bp)

Primer	No. Alleles	olymorphic	Polymorphism %	Allele size (bp)
Ptms-3	3	3	100	129-138-154
Ptms-7	3	3	100	171-182-202
Ptms-9	2	2	100	116-125
Ptms-11	1	0	0	76
Ptms-14	3	3	100	108-113-117
Ptms-31	2	2	100	121-129
Ptms-33	1	0	0	176
Ptms-40	1	0	0	192
Ptms-42	1	0	0	183
Ptms-45	2	2	100	154- 163
EPVF021	6	6	100	101-113-147-307-338-491

EPVF013	2	2	100	633-690
EPVF016	3	3	100	488-550-624
EPVF056	3	3	100	300-375-520
EPVF019	9	9	100	112-120-161-196-202-297-377-532-667
EPVM058	2	2	100	273-294
SUM	44	40	90.91	
AVE	2.75	2.5		

Genetic similarity

The studied genotypes revealed varied genetic distances ranged from 0.24 between both *P.vrea* female cultivars; Ashouri and Batouri (FA and FB) and PA37 bisexual *P. atlantica* genotype, to 0.96 between two F1 progenies HA1 (FA×PA37) and HA2 (FA×PA12).Genetic similarity between Ashouri cultivar and its hybrids was 0.66, whereas genetic similarity between Batouri cultivar and its hybrids was 0.513, (Table 4). Genetic distances between the hybrids and their parents were closer to their female parents (FA and FB) than to their male parents (PA12, PA37 and PA35) except for the HB3 (FB×PA35) which revealed genetic distances 0.37 with FB and 0.43 with PA35. Pazouki *et al* (2010) reported that genetic similarity within *Pistacia* spp. ranged from 0.03 to 0.8.

Table 4. The genetic similarity among all studied genotypes using Jaccard coefficient

	FA	HA1	PA37	PA35	HA2	HA3	PA12	HB1	FB	HB2	HB3
FA	1										
HA1	0.65	1									
PA37	0.24	0.38	1								
PA35	0.26	0.33	0.63	1							
HA2	0.63	0.96	0.41	0.36	1						
HA3	0.64	0.85	0.36	0.35	0.89	1					
PA12	0.28	0.43	0.68	0.59	0.42	0.37	1				
HB1	0.60	0.75	0.33	0.32	0.72	0.74	0.47	1			
FB	0.79	0.54	0.24	0.26	0.52	0.58	0.28	0.67	1		
HB2	0.68	0.53	0.40	0.36	0.57	0.57	0.26	0.54	0.68	1	
HB3	0.30	0.54	0.39	0.43	0.57	0.53	0.34	0.50	0.37	0.42	1

Cluster analysis

The pattern of cluster analysis based on Jaccard’s coefficient and UPGMA algorithm clustered the genotypes into two main clusters (Figure 1); The first cluster divided into three sub-clusters, the first one branched into two groups, the first group contained all F1 hybrids ‘derived from the hybridization between *P. vera* female Ashouri cultivar and all *P. atlantica* bisexual genotypes’ and the second group contained the hybrid HB1 (FB×PA12). The second sub-cluster divided into two groups; The first one contained *P. vera* female cultivars (FA and FB), and the second one contained only the progeny HB2 (FB×PA37). Whereas the progeny HB3 (FB×PA35) was located in the third sub-cluster. All *P. atlantica* bisexual genotypes were grouped in the second cluster which divided into two sub-clusters, the first one contained both of PA12 and PA37, while PA35 genotype was located independently in the second sub-cluster.

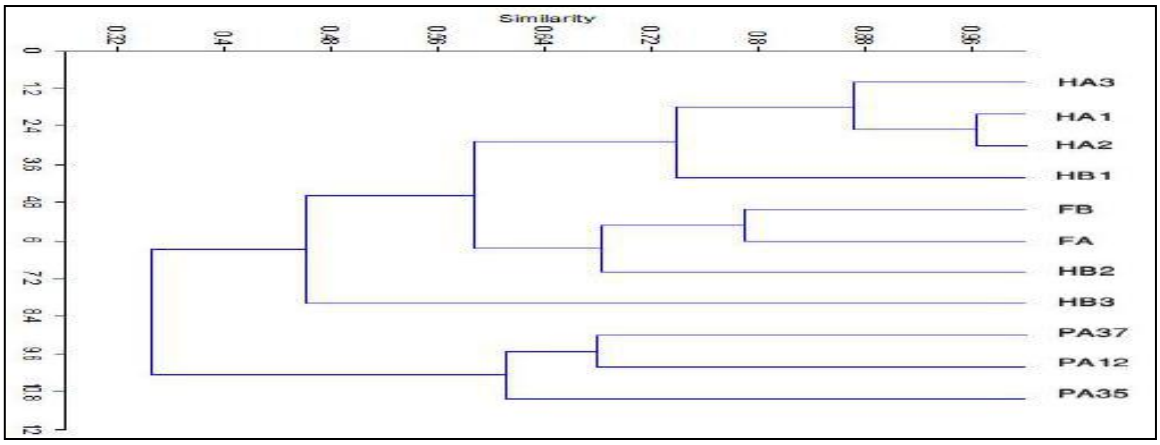

Figure 1. cluster analysis using UPGMA method according to Jaccard's coefficient

To evaluate the efficiency of SSR microsatellite markers for polymorphism detection; Observed and Expected Heterozygosity (Ho and He), and Marker Index (MI) were estimated. Observed heterozygosity (Ho) was appeared in 4 of 16 SSR loci giving an average value 0.25. Expected heterozygosity depending on allele frequency was 0.496 in all polymorphic tested loci. Marker Index (MI) was estimated depending on the effective multiplex ratio which was 19.84. Vendramin *et al.,* (2010) found that Observed Heterozygosity (Ho) was 0.27 while the expected heterozygosity (He) was 0.31 in their study.

Conclusion

The bisexual genotypes PA35 and PA37 were optimized by their high viable and fertile pollen grains ratios, and by giving high nuts set percentage in both cultivars. Our investigation also indicated that SSR marker is an informative technique which revealed high ability to differentiate individuals and played an important role as genetic marker for lineage studies, and may establish a platform to build up an understanding of genetic inheritance in the genus *Pistacia*.

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ASSESSMENT OF GENETIC INTEGRITY OF APPLE SEEDLINGS ROOTSTOCKS DERIVED FROM THE LOCAL APPLE CULTIVAR (SUKARI 2) IN SYRIA USING SSR MARKERS

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Abstract

The local applecultivar Sukari 2 is suitable as a source for seeds due to the desirable traits of its seedling rootstocks, this investigation was aimed to study the genetic similarity among seedling rootstocks and their mother plant (Sukari 2) using 16 primer pairs of SSR markers. The results showed that these primers gave 32 alleles, 25 of them were polymorphic with polymorphism percentage 78.1%, and the genetic similarity between the seedlings and their mother plant was above 0.5 except in one seedling, and the highest value reached to 0.818. Likewise, the highest genetic similarity among seedlings with each other was 0.85. On the other hand, the cluster analysis divided the seedlings into 3 groups, the main group contained the most seedlings with the mother, this high genetic similarity with the mother indicated to the possibility of using the cultivar Sukari 2 as a source for seeds. As well,the expected and observed heterosigosity were 0.37 and 0.48 respectively, this illustrated that the SSR marker is an efficient tool in discriminating among individuals in apple rootstocks breeding program.
Key words: *Local apple cultivar (Sukari 2), seedling rootstock, SSR, genetic similarity*

Introduction

Apple growers just in few countries depend on seedling rootstocks, only in the countries which are difficult there to use vegetative rootstocks, or they are not economical (Webster and Wertheim, 2003). However, seedling rootstocks have many advantages over vegetative rootstocks, that they are easy and cheap in propagation, virus free, and free from soil rots which infest roots. Moreover, it is possible to eliminate wide genetic diversity through using defined sources for seeds such the cultivar Red Delicious in America, the cultivars Antonovka and Bittenfilder in Europe, and the wild species in China and Japan (Wertheim and Webster, 2003).In Syria, a group of apple genotypes was selected as a candidate sources for seed production (Al-Halabi et al., 2012a), the local apple cultivar (Sukari2) is one of this group, that the seedling rootstocks derived from it are similar to each other in vigor and diameter, in addition they have the desirable rootstock traits according to Cummins and Aldwinckle (1995). Molecular markers have been proved high efficiency in identifying apple rootstocks in comparison with morphological characters (koc et al.,2009). However, SSR markers which were used to discriminate among genotypes derived from different apple rootstocks (Oraguzie et al., 2005). The genetic similarity among 14 genotypes of apple rootstocks which were produced by the hybridization between the local apple cultivar (Skarji) and MM106 rootstock was achieved using SSR markers (Al- Halabi et al., 2012b). On the other hand, SSR markers were used to study genetic diversity among 41 apple rootstocks from dwarfing, semi dwarfing rootstocks and rootstocks of early flowering*Malus.* Sp (Jin et al.,2012). Our research aimed to evaluate genetic similarity among seedling rootstocks derived from local apple cultivar (Sukari 2) using SSR markers.

Materials and methods

The present investigation was carried out at the agricultural scientific research center – GCSAR- in Sweida province, which is located at 1525m altitude in the south of Syria, during 2010.

Plant material

10 seedling rootstocks derived from the local apple cultivar (Sukari2).

DNA extraction

DNA extraction was achieved using CTAB protocol according to Porebski et al., (1997), by collecting leaves from the seedling rootstocks, in addition to the mother plants (Sukari2).

PCR amplification

PCR amplification was achieved using 17 primer pairs are developed from apple, 10 primer pairs (Hi-SSR) were developed by Silverberg et al. (2006), 6 primer pairs (CH-SSR) by Gianfranschi et al. (1998), and one primer pairs (o2b1) by Guilford et al., (1997).

The reaction was performed with volume (10 µl) consisted of: 1 µl 10 X buffer + 1 µl dNTPs + 1 µl forward primer + 1 µl reverse primer + 3 µl DNA + 0.1 µl taq + 2.9 µl dH_2O. The cycling profile consisted of an initial denaturation step of 3 min at 94º c, followed by 35 cycles of 30 s at 94ºC, 30 s at 55º -58º C (according to primer pairs and its content of CG) and 1min at 72ºC, the amplification process was finished with 5 min at 72ºC.

Visualization of the PCR products

The PCR productswere detected by electrophoresis on 2% agarose gel in 1X TBE buffer, stained with ethidium bromide and visualized by UV light and photographed using gel doc. The molecular weight of the bands was estimated by Bioprofil- Bio- 1D software using 100bp DNA Ladder.

Data analysis

genetic similarity (GS) was estimated according to Jaccard coefficient (Jaccard, 1908), that all bands were recorded and the present band was given 1 and the absent one was given 0.Cluster analysis achieved using UPGMA method (unweighted pair-group method using arithmetic averages) by Past software. Expected Heterozygosity (He) was calculated according to Lorenzo et al, (2007). While the observed Heterozygosity (Ho) was calculated according to Wunsch and Hormaza (2007).

Results and discussion

The polymorphism

16 primer pairs revealed 32 alleles which were 25 ones of them polymorphic, with polymorphism percentage 78.1%, while the primer pair Hi07f01 could not reveal any allele. The number of alleles in different loci was between 1-3 alleles, with an average 2 alleles per locus. This result was in agreement with the result obtained by Farrokhi et al. (2011) in their study of genetic diversity among old Iranian apple cultivars using 16 SSR primer pairs, which revealed 2-5 alleles per locus, indicated that this result is closer to that one obtained from self pollination plants such tomato, cucumber and wheat. However, each of CH01H01, Hi08g06 and Hi08e06 primer pairs gave monomorphic alleles. On the other hand, 13 primer pairs were able to detect the genetic variance among studied seedlings, that the most primer pairs showed two polymorphic alleles (Figure 1) except in CH01E01, CH02B12 and O2b1 which revealed one monomorphic allele and the other was polymorphic. While, Hi04g05, CH01H02 and CH01F02 gave 3 alleles. Likewise, the co-dominant appeared in the mother plant (Sukari2) at the loci CH01F02, CH02B03b, CH01E01 and CH02B12 which indicated that the local apple cultivar (Sukari 2) is a hybrid, this result was in agreement with Al-Halabi et al. (2009). The

size of the obtained alleles was between 86 bp in CH02B03b and 265 bp in Hi07h02, which was in agreement with the predictable size according to Guilford et al., (1997); Gianfranschi et al. (1998) and Silverberg et al. (2006).

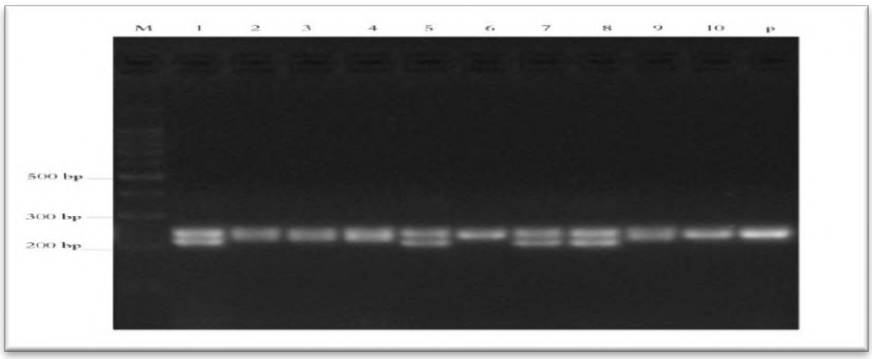

Figure (1): The alleles patterns produced by the primer pair O2b1 in the studied seedlings and the mother plant (Sukari 2). M: Marker 100bp ladder, the numbers from 1 to 10 represents seedlings, and P: the mother plant (Sukari 2).

Genetic similarity

The studied seedlings showed closer genetic distance with the mother plant (Sukari 2), it was above 0.556 except in seedling 4, which was 0.423, while the highest genetic similarity value was 0.818 with seedling 9. On the other hand, the highest genetic similarity value among studied seedlings was 0.85 between seedling 2 and seedling 3, and the lowest one was 0,296 between seedling 4 and seedling 5. Theseresults were in agreement with Al- Halabi et al. (2012b) they found that the highest genetic similarity value among seedlings derived from hybridization between MM106 and local apple cultivar Skarji using SSR markers was 0.84, and the lowest one was 0.29. The seedlings 1, 7 and 9 distinguished by the high genetic similarity in comparison with the other seedlings, which was above 0.5 except seedling 4 (Table 1). These results indicated to the high genetic similarity among the seedlings derived from the local apple cultivar (Sukari 2).

Table 1. Genetic similarity among the studied seedlings and the mother plant (Sukari 2) derived from SSR markers depending on Jaccard coefficient.

	1	2	3	4	5	6	7	8	9	10	Sukari2
1	1.000										
2	.560	1.000									
3	.667	.850	1.000								
4	.407	.400	.333	1.000							
5	.696	.800	.762	.296	1.000						
6	.625	.714	.762	.400	.636	1.000					
7	.640	.727	.696	.423	.652	.727	1.000				
8	.720	.481	.464	.560	.538	.481	.680	1.000			
9	.708	.727	.773	.423	.652	.810	.739	.556	1.000		
10	.577	.652	.625	.609	.520	.583	.600	.500	.667	1.000	
Sukari2	.708	.652	.773	.423	.583	.727	.739	.556	.818	.667	1.000

Cluster analysis

Cluster analysis divided the studied seedlings into three clusters, the first cluster contained 7 seedlings in addition to the mother plant (Sukari 2), this clusterdivided into 3 sub clusters, the first one involved 3 seedlings, the second sub cluster contained 3 seedling with the mother plant, while the third one has only one seedling. The second cluster involved two seedlings, while the third clusterhad only one seedling.However, cluster analysis divide the studied plant populations according to their pedigrees (Hormaza, 2002; Muzher, 2004; Perez et al., 2005; Amirbakhatiar et al., 2006).Genetic diversity in apple is mostly belonging to cross pollination

(Janick et al., 1996), but the local apple cultivar (Sukari 2) has an early flowering stage before all other cultivars (Al-Halabi et al. 2009) , which increases the chance of self pollination, thus lead tohigh genetic similarity with its progeny.

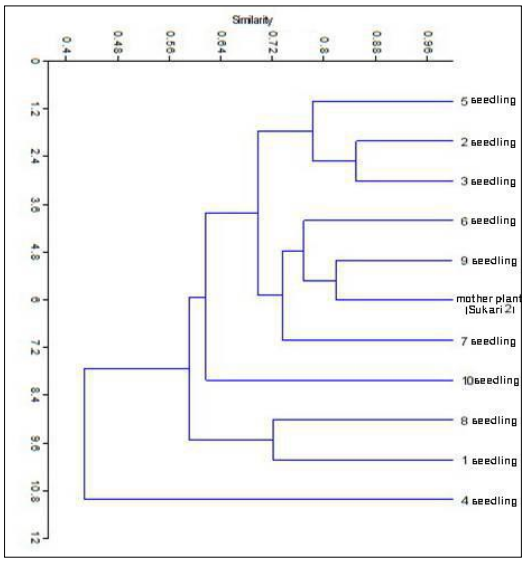

Figure 2. Cluster analysis dendrogramderived from SSR marker , depending on Jacard coefficient using UPGMA method for the seedlings and their mother plant (Sukari 2).

Expected and Observed Heterozygosity

The results showed that the expected heterozygosity (He) derived from SSR markers within the studied seedlings was 0.37, while the observed heterozygosity (Ho) was 0.48. However, He and Ho are useful in determining the genetic variability, that these values were lower than that in the literature reviews, which indicated to the low genetic variability among seedlings derived from the local apple cultivar (Sukari 2), this led to the probability of using Sukari 2 as a source for seed production. The He value in this research was in agreement with the He obtained by Muzher and Sharaf (2014) in theirstudy of genetic variability and relationship among Syrian pears and wild type genotypes using SSR markers which was low0.263. while it was in disagreement with Guarino et al. (2006) that the He was between 0.713 and 0.884 within some of apple cultivars. The characterization of old apple cultivars in comparison with 11 standard cultivars using SSR markers showed that the He and Ho in the old cultivar were 0.82 and 0,84 respectively, while in the standard cultivars were 0.9 and 0.79 respectively which reflected that the cross pollination in apple is high, which consequently lead to the genetic variability (Sikorskaite et al., 2012).

Conclusion

The low polymorphism percentage (78.1%), expected heterosigosity (0.37) and observed heterosigosity (0.44),then the high genetic similarity and the cluster analysis which were obtained using SSR markers indicated to the low genetic variability between the seedlings and their mother plant the local apple cultivar (Sukari 2).Consequently, our results can suggest to use the local apple cultivar (Sukari 2) as a source for seed production, on the other hand SSR markers are a useful tool in apple breeding program.

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MAIZE BREEDING FOR QUALITY TRAITS

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Abstract

Grains of cereals are a main resource for human nutrition and animal feed throughout the world. At Maize Research Institute programs for improvment quality of protein, and micronutritiens as beta carotine, tocopherols and minerals are conducted. In the case of biofortification nutritional breeding also starts with assembly of parental germplasm for crosses based on the evaluation of a large amount of genetic material. More than 100 inbred lines are screened for concentration of microelements as well as beta carotene and tochopherols. A high amount of variation for micronutrients is present among genotypes and genotypes with high concentration of micronutrients are chosen for further crossing. Maize grains have particularly high concentration of phytate which negatively impact human and animal nutrition. Inorganic phosphorus is forms of P in seeds that generally referred as available P. Therefore, it would be desirable to increase the amount of available P and reduce the amount of phytate in maize grain. Among analyzed maize inbred lines are enough variability for phytate and ionrganic phosphorus that can be used for breeding maize genotypes with low phytate and high inorganic phosphorus content and good agronomic traits. Maize lacks adequate levels of the essential amino acids lysine and tryptophan, thus reducing the overall biological value of its protein. Quality Protein Maize (QPM) is argonomically acceptable and nutritionally improved *opaque2* maize, with increased levels of tryptophan and lysine in the grain. At Maize Research Institute project on conversion of normal maize lines to QPM lines adapted for growing in temperate regions trough MAB is developed.

Key words: *maize, QPM, micronutritiens, biofortification, quality traits*

Introduction

Maize is a high yielding crop that provides a relatively high calorie source of food and feed, but focus on yield has not improved nutritional qualities. Some nutrients that are lacking in maize are beta carotene, iron and essential amino acids. A variety of breeding methods can be used to improve qualities of maize. Conventional approaches include screening of germplasm, mutagenesis, and hybridization. Those traditional methods can be complemented with biotechnology approach such is transgenesis, cisgenesis, overexpression or suppression of gene of interest. The use of molecular biology to isolate, characterize, and modify individual genes followed by classical breeding strategy or plant transformation and trait analysis will introduce new traits and more diversity into maize database. Backcrossing can be used to bring a mutation or transgene into a specific genetic background, while forward breeding can be used to improve characteristics of a line that carries a gene of interest. Biofortification with selective breeding or biotechnology is a way to improve the nutritional qualities of crops. Finding genes which control accumulation of minerals in maize grains is the precondition for biofortified breeding program. Maize and relatives contain many genes that have the potential to improve nutritional quality of maize grain, as maize globin for bioavailable iron and seed storage proteins for protein quality. Few QTL mapping researches have also been conducted on micronutrient content in maize (Qin *et al.* 2012, Šimić *et al.* 2012); however, the results were inconsistent possibly due to the differences in mapping populations, genotypes and

environments used in these studies. Kobayashi and Nishizawa (2012) have shown that the Zn and Fe metabolism is a complex process regulated by many genes. Development of maize with unique grain quality traits, however, has not received the same emphasis in genetics, breeding, and economics as higher yield and agronomic performance. Enhanced grain protein and micronutrient content for both vitamins and minerals is rapidly emerging as the next suite of seed quality traits to be improved.

Improving of maize protein quality

The most well-known experiment that altered total protein content in maize is the Illinois Long Term Selection Experiment. In these studies it was shown that protein content could be increased up to 26.6 percent in the high-protein (HP) genotype after 65 generations of selection. The low-protein genotype contained about 5.2 percent (Moose et al, 2004).

Maize protein is deficient in two essential amino acids, lysine and tryptophan. Therefore, maize is a poor source of protein for both humans and monogastric animals. Lysine, along with methionine and tryptophan, are important targets for improvement of maize protein quality. While there is little variation in amino acid content in modern inbred lines, some mutations such as *opaque2* significantly alter maize amino acid composition. O*paque2* is a transcription factor that controls expression of zeins and of an enzyme that degrades free lysine. Maize with the *opaque2* mutation has decreased zeins and higher free lysine. The *opaque2* (*o2*) mutation which increases lysine and tryptophan content also confers an undesirable phenotype leading to low yields, soft and chalky kernels that renders seeds susceptible to storage pest and ear rots. Using conventional breeding methodologies, research team in CIMMYT, Mexico, created the new, agronomically acceptable and nutritionally improved *opaque2* types by the name of Quality Protein Maize or QPM (Vivek *et al*., 2008).

Research on *opaque2* mutation and high tryptophan was initiated in the 1970-ies in Maize Research Institute Zemun Polje - MRI (Denic *et al*., 1979). Today's MRI breeding program on conversion of normal maize lines to QPM lines adapted for growing in temperate regions trough marker assisted backcross breeding. are relate to: 1) genetic and biochemical variability analysis between one commercial normal maize inbred line and one QPM inbred line in order to be used as parents in marker assisted selection for quality protein maize and 2) efficiency of the three *opaque2* specific SSR markers and the choice of appropriate markers for marker assisted introgression of the *opaque2* gene into the commercial normal inbred line, (Denic *et al.*, 2012; Ignjatovic-Micic *et al*., 2010, 2013). The main goal is to achieve high levels of essential amino acids and high grain yield at the same time. The donor line was CML144, one of the CIMMYT Maize Lines (CMLs) with good general combining ability and a significant number of value-adding traits such as drought tolerance, enhanced levels of the essential amino acids lysine and tryptophan, N use efficiency, acid soil tolerance, resistance to diseases, insects and parasitic weeds. ZPL 5, dent-like commercial MRI inbred lines adapted to the local environmental conditions was selected as the recurrent parent for marker assisted backcross programme to be the recipient of the *o2* allele and to improve the tryptophan content. SSR markers were effectively employed both for selecting *o2* homozygous recessive genotypes and genotypes with the highest proportion of recurrent parent genome. Recipient parent and donor lines were clearly distinguishable with three *opaque2*specific SSR markers (Kostantinović et al, 2014). Therefore, they will be used for marker assisted selection for the *opaque2* gene. Marker analysis will be done using DNA samples extracted from leaf tissue of very young plants. This enables those plants without *o2* alleles be discarded prior to pollination, reducing the size of the breeding population and saving both time and money. Due to the well-established relationship between lysine and tryptophan in the maize protein (4:1 ratio), tryptophan can be used as a single parameter for evaluating the nutritional quality

of the protein. CML 144 had tryptophan content and quality index higher than ZPL 5 and both above threshold set for QPM.

The process of conversion of the standard inbred line in line with improved proteins quality using molecular markers consisted of two cycles of backcross which included three cycles of self-pollination (Figure 1). Molecular analyzes were carried out in the BC1, BC2 and BC2F2 generations for the determination of heterozygosity / homozygosity for opaque2 gene, and BC2, BC2F3 and BC2F4 generations, as well as improved lines for determining the similarity of progeny with the recurrent parent. Phenotypic selection included the biochemical evaluation (determination of tryptophan, protein content and quality index - the percentage of tryptophan in the protein), determining the degree of modification of the grain endosperm and evaluation of important agronomic characteristics. Inbred lines with increased tryptophan content (37-50% compared to the parental line), held genetic similarity to the parental line (93% of the genome of the recurrent parent), increased grain yield (11-31% compared to the parental line) and preserved combining abilities are obtained.

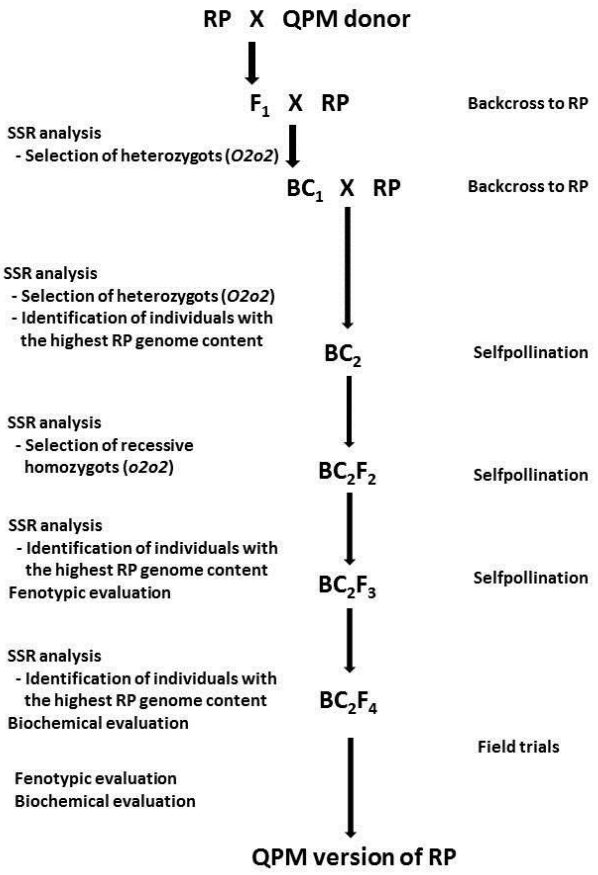

Fig 1. Scheme of standard lines conversion (RP - the recurrent parent) in QPM version.

Improving micronutrients content

Although maize is low in some micronutrients, humans and animals can obtain at least part of their nutritional requirements from maize grain. Biofortification is the use of crop breeding practices and/or modern biotechnology to produce micronutrient-dense staple crops. The conventional approach to mineral biofortification has been to work at three levels. These are to increase the density of the mineral nutrient of interest, to decrease the density of antinutritive compounds (nutrient inhibitors), and to increase the density of compounds that enhance bioavailability of the specific nutrient.

In Maize Research Institute we develop program that aimed to improve micronutrient content and bioavailability. In any breeding program, germplasm screening for a trait of interest is an important first step to genetic improvement. In the case of biofortification, nutritional breeding also starts with assembly of parental germplasm for crosses based on the evaluation of a large amount of genetic material. Drinic et al (2013) studied the variability of microelements in grain of 74 maize inbreed lines. The highest average concentration of Mg, Fe and P had inbred lines from European germplasm while inbred lines from BSSS germplasm had the highest Zn concentration. Lines from BSSS had the lowest average Mg and Fe content as well as lines from Lancester germplasm had the lowest Zn and P content. Some studies have shown that there is sufficient genotypic variation in major cereal crops to breed for higher total iron (Zhao and McGrath 2009), but an evaluation of more than 1000 improved maize genotypes and 400 maize landraces found little variation of total iron in the grain (Ortiz-Monasterio et al. 2007).

The main iinhibitor of absorption of iron (Fe), zinc (Zn), and magnesium (Mg) from cereal grain is phytate (for review,White and Broadley 2009). Dragičević et al (2013) obtained high variability in concentration of Fe, Mn and Zn, as well as phytate (which sustain availability of mineral elements) and β-carotene (which enables better absorption of mineral element and minimize negative effect of phytic acid) in maize inbred lines. Kravic et al (2014) find out that investigated drought tolerant maize local landraces exhibited high variability in concentration of important mineral elements, phytic acid and β-carotene. According to relatively high concentrations of all four factors observed (β-carotene, Fe, Mn and Zn), as well as the lowest ratios between Phy and all examined mineral elements, drought tolerant maize local landrace LL2 is the most promising genotype in further breeding programs for improved nutritional quality, such as enhanced availability of investigated mineral elements. A high amount of variation for micronutrients is present among investigated maize genotypes and genotypes with high concentration of micronutrients are chosen for further crossing. Part of our research is aimed to decrease phytate and increase inorganic phosphorus content. In breeding for increased concentrations of carotenoids and tocopherols, it is also important to know what effect male and female genotypes contribute to kernel content for these compounds when different genotypes are crossed (Drinic et al., 2014).

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THE RESPONSE TO STOMATAL CONDUCTANCE AND CHLOROPHYLL VALUE OF GROUNDNUT GROWN UNDER SALT STRESS IN DIFFERENT DEVELOPMENT STAGES

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Abstract

In this study the response of groundnut to salinity over different development periods (blooming, ginofor formation and fruit formation and maturation) was determined in the Eastern Mediterranean Region. The experimental design was split plot in a randomized complete block with three replications and was conducted in a total of 180 pots. In the irrigaton of groundnut, saline water with electrical conductivity (ECw) 0.19 dS m^{-1} ($T_{0.19}$), 3.54 dS m^{-1} ($T_{3.54}$), 7.12 dS m^{-1} ($T_{7.12}$) and 12.86 dS m^{-1} ($T_{12.86}$) were used.

It was determined that Ginofor formation stage was the most sensitive stage to salinity than the other developmental stages. Yield parameters were affected at $p < 0.001$ level from *development stage, irrigation water salinity and the development stage x irrigation water salinity* interaction. Chlorophyll values at different salinity levels of stoma conductivity was show that different. The highest and lowest stomatal conductance were realized in $T_{3.54}$ (356.025 mmol m^2s^{-1}) and $T_{12.86}$ (238.25 mmol m^2s^{-1}) issues. Stomatal conductivity showed differences at different salinity levels. The highest and the lowest stoma conductivity monitored at $T_{3.54}$ (356.025 mmol m^2s^{-1}) and $T_{12.86}$ (238.25 mmol m^2s^{-1}). It was observed that the at the plots where the highest stoma conductivity determined had the higest yield.

Key Words: *Groundnut, salinity, development periods, stomatal conductance, chlorophyll*

Introductıon

Drought problems arising in the arid and semi-arid regions makes almost mandatory to use of marginal quality water in irrigation (brackish, reclaimed, drainage and waste water). Saline water usage causes the salinization to about 830 million hectares of worldwide (Martinez-Beltran and Manzur, 2005). Because of this situation, it has become necessary to determine the salt water-yield function in salty field in detail (especially for the plants that play an active role in human nutrition).

One of plant species rich in oil, protein, carbohydrates, vitamins and mineral substances is groundnut (Arıoğlu et al., 2010). Worldwide about 45.6 million tonnes in 100 countries, groundnut production is realized (FAO, 2013). Yield losses due to drought may vary with time and associates with temperature and high stress factors, such as region-specific irradiance. About 2/3 in arid regions, crop production potential is limited , to the seasonal rainfall. In these areas, productivity changes between 0.7 - 0.8 Mg ha^{-1}. But, even with that limited water efficiency in commercial areas are level of 2.0-4.0 Mg ha^{-1} (Smartt, 1994). Lack of soil moisture in groundnut farming and irrigation water quality are considered to be the most important factors limiting the yield. Growth period changes from about 120 to 150 days. precipitation of 500-700 mm is sufficient for the cultivation of groundnuts during the growth. But this fall should be distributed to the growing period. Groundnut maximum daily water consumption occurs in flowering, pod-linking and maturation. The highest water consumption is in July-August, In August-September, it was measured as about 6.0-8.5 mm day^{-1}. In starting period (germination) when the groundnut needs adequate soil moisture, small degree of water stress should be applied a during the vegetative period. Flowering period is the the

most sensitive stage to water shortages. In general, extreme water shortages in the vegetative period causes delay of flowering and the formation, development and harvesting of fruit. Water shortages of the flowering period lead to loss of flowering and poor flower pollination. Studies conducted in response to the saltwater applications of groundnuts is almost negligible. To our best knowledge, revealing different stages of plant development functions of salt-yield research does not exist in literature.

In this research, the response of groundnut during three different development to different irrigation water salinity effects of yield, vegetative and physiological parameters were determined.

Material and method

Research was conducted in greenhouses and sheltered from rain, between May and October 2012, using NC-7 variety of the Virginia group (leaning early). The plants were grown in 43 cm diameter and 47 cm high plastic pots. Each pot filled with sandy-clay loam soil (42.0% sand, 35.3% clay, 22.7% loam) which has a volume weight of 1.38 gr cm^{-3}, salinity C_1S_1.

The experiment was conducted according to the split plot in a randomized complete block design in three different growth period (Blooming, Ginofor formation, The formation and maturation of fruits periods), 4 different salinity in irrigation water (ECw) (0.19 dS m^{-1} (T_0), 3.54 dS m^{-1} ($T_{3.5}$), 7.12 dS m^{-1} (T_7) ve 12.86 dS m^{-1} (T_{13})). The experiment was repeated three times and total number of pots were 180. NaCl salt and pure $CaSO_4$ salt sources were used in the experiment. Na and Ca values in the irrigation water were kept between 0.1 and 0.7 (Grattan ve Grive, 1999). Prior to each irrigation, extra 3 post were used in the determination of the amount of irrigation water. 20% of leaching water was applied at each irrigation treatment to issues except for T_0. Irrigation water salinity (dS m^{-1}) was measured by portable EC meter (Orion 3 Star, USA), soil moisture content (cm^3 cm^{-3}) and soil salinity was measured by ΔT marka HH-2 moisture meter. Before the experiment, calibration equation was determined for the soil salinity y= 0.0127x + 0.91, (r^2=0.96**). In order to determine the evapotranspiration, , 3 pots were weighed prior to each irrigation. The weighed differences between the pots at each irrigations were summed and the seasonal evapotranspiration were calculated. During harvest, the plants were removed from the pots and the soil was removed and then the groundnut yield was determined. The physical characteristics of grains were measured as width (mm), length (mm), the average grain weight (gr) and numeral (number m^{-2}). Plant height were measured to determine the vegetative response before each irrigation.

In this research, stomatal conductance and chlorophyll content were read a total of 6 times in 2 pots out of every replication before irrigation in order to determine the effects of irrigation water salinity on plant physiology. Stomatal conductance was measured by porometer (Model SC-1. LPS0881) and chlorophyll content was measured by Minolta SPAD 502. Stomatal conductance and chlorophyll content were measured in the middle of parcel, the full-blown fresh leaf which see the sun, In outdoor conditions between 12:00-14:00 o'clock, once a week and before irrigation.

Result and dıscussion

Soil Moisture Content, Soil Salinity and Evapotranspiration: In this research, the plants were irrigated 4 times in blooming and ginofor period and 3 times in the fruit ripening period. Unsalted water for irrigation has been applied to all treatments from planting to exit from to soil period. Salt water applications have started blooming period and continued until the harvest period. Soil salinity has increased significantly depending on irrigation practices (except forT_0). 0.19 dS m^{-1} irrigation water even did not cause a significant reduction in salinity level in the next period of saltwater applications in blooming and ginofor formation periods. This situation is due to the presence of Na ions in the soil being heavily complex and

the high hydration diameter do not impede the full realization of the leaching process (Frenkel ve ark. 1978). Evapotranspiration (Et) decreased overall by increasing salinity (Table 1). The highest Et was measured in control treatment in blooming and ginofor formation periods and it also was measured in $T_{3.54}$ issue in during fruit ripening period. Significant relationship was found (y=-14.307x+799.15 r^2=0.95**) in blooming period between Et and average ECe, and also in ginofor formation periods (y=-10.081x+754.35, r^2=0.93**) and insignificant relationship was found (y=-12.357x+745.34 r^2=0.74ns) during fruit ripening. Increased salinity in 1 unit of dS m^{-1} of 1 causes a decrease 14.3 mm of Et in blooming period and also in ginofor formation period it causes a decrease 12.35 mm.

Table 1. The average values of evapotranspiration and soil salinity in the growth period

Treatment	Blooming Period		Ginofor Formation Period		Fruit Formation and Maturation Period		Seasonal Average	
	Et (mm)	ECe (dS m^{-1})	Et (mm)	ECe (dS m^{-1})	Et (mm)	ECe (dS m^{-1})	Et (mm)	ECe (dS m^{-1})
$T_{0.19}$	813.00	1.15	744.00	1.62	714.33	0.62	757.11 a	1.13
$T_{3.54}$	738.00	3.36	716.67	2.45	757.33	1.67	737.33 a	2.48
$T_{7.12}$	677.67	6.88	704.33	4.38	630.00	2.88	670.67 b	4.69
$T_{12.86}$	628.67	12.61	613.33	8.25	586.67	4.57	609.56 c	8.42

Stomatal conductance, Chlorophyll Fluorescence: Increase in irrigation water salinity decreased the stomatal conductance (Figure 2). Average of stomatal conductance was measured respectively in T_0, $T_{3.5}$, T_7 and T_{13} as 307.476, 356.025, 268.187, 223.056 mmol m^{-2} s^{-1} .According to Duncan analysis, stomatal conductance values of $T_{3.5}$ and T_{13} were took place separate groups and also T_0 ve T_7 were same. The stomatal conductance of $T_{3.5}$ was determined higher than the T0 (control). This was evaluated as the final effort of the plant to recover from the salt stress. The regression coefficient between stomatal conductance and soil salinity showed different linear relationships in the blooming period (y=-2.43x+340.l63 r^2=0.12 ns), in ginofor formation period (*y=-34.484x+418.56 r^2 = 0.96***), also in fruit formation and maturation period (y =-18.417x+310.3 r^2 = 0.37ns) As seen, the effect of soil salinity on stomatal conductance during ginofor formation more important than the other periods. The highest and lowest stomatal conductance in growth period were measured in blooming (326.053 mmol m^{-2} s^{-1}) and fruit formation periods (265.440 mmol m^{-2} s^{-1}).

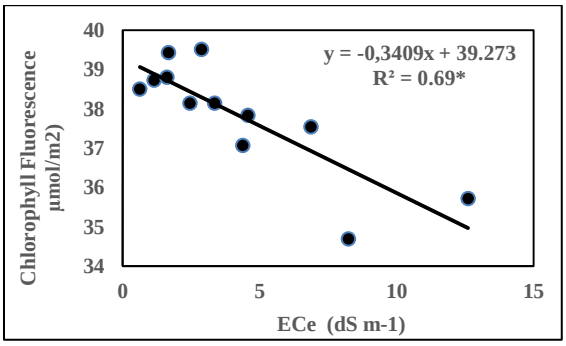

Figure 2. The relationship between chlorophyll content and soil salinity

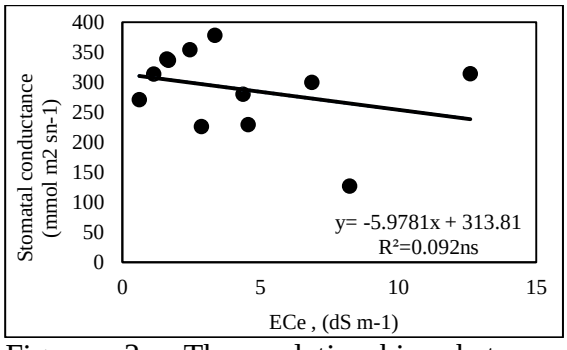

Figure 3. The relationship between stomatal conductance and soil salinity

Average values of ECe were measured between 5.99 dS m^{-1} and 2.43 dS m^{-1} in the same period. Despite the increased stomatal conductance reduction of soil salinity may be a result of cause aging of salinity stress in plants during the growth period. The effects of soil salinity on stomata conductance ($p<0.01$) was the more efficient than growth periods has been

determined in statistical analysis (Table 2.). The highest regression coefficients were determined in the ginofor formation period regression analysis between stomatal conductance and ECw ($p<0.05$).

Table 2. Variance Analysis of the Physiological Parameter

Variation Source	Stomatal conductance (mmol m^{-2} s^{-1})			Chlorophyll Fluorescence (µmol m^{-2})		
	Sd	KO	F	Sd	KO	F
ECe	3	260406.817	3.357*	3	151.341	7.469**
Growth stages (Gs)	2	125428004	1.617ns	2	111.210	5.489**
ECe* Gs	6	58409.788	0.753ns	6	22.327	1.102ns
Corrected Total	344					

Gs: Growth stages, ECe: electrical conductivity of soil paste (dS m^{-1}), Sd:Degree of freedom

Chlorophyll Fluorescence: Chlorophyll values decreased due to the increase in salt concentration (Figure 3). However, this reduction was not statistically significant (r^2=0.89ns, $p<0.05$). Chlorophyll values of control treatment ($T_{0.19}$) was measured 38.674 µmol m^{-2}, and also in $T_{13.86}$ 36.080 µmol m^{-2}. Chlorophyll values of $T_{3.5}$, T_7 were determined respectively, 38.569 µmol m^{-2} and 38.038 µmol m^{-2}. $T_{0.19,}$ $T_{3.5}$, T_7 issues were included in the same group, $T_{12.86}$ was icluded in the different group. The lowest chlorophyll value during the period was measured in $T_{12.86}$ by 35.193 µmol m^{-2} and also the highest value was measured in $T_{0.19}$ by 39.491 µmol m^{-2}. The effect of soil salinity on chlorophyll value at each development cycle were different. In regression analysis between soil salinity and chlorophyll content was obtained as y=-0.2588x+39.084 R^2=0.99** in blooming period, y=-0.61x+39.721 R^2 = 0.99** in ginofor formation period and y=-0.1948x+39.29 R^2=0.17ns in fruit formation and maturation period. As it is seen from the equations, closing to the harvest time, chlorophyll values of the plants became more erratic than the other periods. In addition, to the weakening synthesis of chlorophyll towards the end of the the plant's life cycle and entering senescence salinity stress is a major cause of this condition. Statistically, chlorophyll value was significantly affected by developments periods and soil salinity (*$p<0.01$*, Table 2). While chlorophyll values in blooming an ginofor formation periods located in the same group (37.531 µmol m^{-2} and 37.174 µmol m^{-2}), fruit formation and maturation period were located in a different group (38.815 µmol m^{-2}).

The Relationship Between Physiological Characteristics and Yield: The response of physiological parametres of Groundnut to the irrigation water salinity was shown with analysis of variance in Table 3. The analysis of variance shows that yield parameters were affected at the level of *$p<0.001$* in *their growth period, irrigation water salinity and interaction of growth period x irrigation water salinity.* When the average value of the yield parameter in the development period analyzed, highest values were obtained from fruit formation and maturation periods. This situation shows that if the saline water implemented closer to harvest in the growth period, it would be relatively lower average yield reduction. Stomatal conductance and chlorophyll values at each growth period were not effective at the same level. When regression relationships are examined, both physiological properties were determined to be most effective in ginofor formation (Table 3).

Table 3. The stomatal conductance and Chlorophyll fluorescence parameters relationships with yield (Regression Equations)

		Lineer regression					Polynomial regression				
		Number of groundnut (number/m^2)	Grain W.(gr)	Width (mm)	Length (mm)	Plant Height (cm)	Number of groundnut (number/m^2)	Grain W.(gr)	Width (mm)	Length (mm)	Plant Height (cm)
Sc (mmol m^{-2} s^{-1})	Blo	y=-0.0144x +84.31 R^2 = 0.001	y=0.1009x +60.706 R^2 = 0.16	y=0.0606x +76.164 R^2=0.24	y=0.0446x +82.675 R^2=0.37	y=0.1797x +31.156 R^2=0.23	y=-0.023x^2 +15.9x-2615 R^2=0.60	y=0.0083x^2-5.5449x+1015.6 R^2=0.36	y=0.0066x^2-4.44x+837.36 R^2=0.77	y=0.0035x^2-2.32x+483.06 R^2=0.80	y=0,0068x^2-4.447x+813.7 R^2 = 0.29
	GNF	y=0.369x -27.985 R^2=0.81	**y=0.2955x -1.3599 R^2=0.99****	**y=0.1385x +52.67 R^2=0.97****	**y=0.0997x +65.818 R^2=0.99***	y=-0.0097x +100.86 R^2=0.53	**y=-0.0034x^2 +1.9957x-187.08l R^2=0.99**	**y=-0.0006x^2 +0.5752x- 28.714 R^2 = 0.99****	**y=-0.0005x^2 +0.37x +29.817 R^2=0.99****	**y=-0.0002x^2 +0.1776x+58.20 R^2=0.99****	y=0.0002x^2-0.0845x + 108.18 R^2 = 0.89
	FFM	y=0.0668x +76.202 R^2=0.14	y=0.0918x +68.836 R^2=0.37	y=0.0289x +90.638 R^2=0.33	y=0.0132x +95.051 R^2=0.15	y=-0.0312x +104.54 R^2 = 0.27	y=-0.0013x^2 +0.7786x-20.952 R^2=0.17	y=0.0005x^2-0.1909x+107.42 R^2=0.38	y=-0.0006x^2 +0.364x+45.382 R^2=0.43	y=8x$10^{-5}$$x^2$-0.033x + 101.39 R^2=0.15	**y=-0.0018x^2+0.9642x-31.325 R^2 = 0.93***
Chll Fluor. (µmol m^{-2})	Blo	y=5.054x -110.07 R^2=0.20	**y=6.489x -149.93 R^2 = 0.90***	y=2.4099x +5.49 R^2=0.52	y=1.4074x +4.384 R^2=0.51	**y=9.910x -282.1 R^2= 0.95****	**y=12.278x^2 -906.9x+16806 R^2 = 0.99****	**y=-2.5579x^2 +196.47x-3673.9 R^2=0.99****	**y=-2.6925x^2 +202.39x-3704 R^2 = 0.94***	y=-1.4382x^2 +108.2x – 1937 R^2=0.86	**y=-1.3126x^2+107.4x-2090.5 R^2 = 0.96***
	GNF	y=21.055x-709.35 R^2 = 0.74	**y=16.742x - 542.58 R^2 = 0.96***	**y=7.6992x -195.51 R^2 = 0.90***	**y=5.646x-116.7 R^2=0.96****	y=-0.595x +120.31 R^2=0.59	**y=-8.3659x^2 +634.34x-11927 R^2 = 0.94***	**y=-3.2268x^2 +253.3x-4869.2 R^2 = 0.99****	**y=-2.3225x^2 +177.9x-3309.6 R^2 =0.99****	**y=-1.037x^2 +81.6x - 1507.1 R^2 = 0.99****	y=0.2266x^2 -17.21x + 424.19 R^2 = 0.69
	FFM	y=9.671x -281.45 R^2 = 0.69	y=9.0227x -257.02 R^2=0.87	y=2.394x +5.3736 R^2=0.54	**y=2.1102x +16.652 R^2=0.90**	**y=-1.1442x +140.68 R^2=0.09**	**y=-15.516x^2 +1210.7x-23516 R^2=0.97**	**y=-7.7912x^2 +612.13x-11925 R^2=0.98**	**y=-5.5544x^2 +432.3x-8312.4 R^2=0.99**	**y=-1.6864x^2 +132.6x-2508.8 R^2= 0.99****	y=-6.1588x^2 +475.6x-9082.3 R^2 = 0.48

** Blo: Blooming stage, GNF: Ginofor Formation Stage, FFM: Fruit Formation and Maturation Stage

Chlorophyll values were effective on a greater number of yield parameters according to the stomatal conductance. In particular, grain weight, grain width, grain size were an important relationship with stomatal conductance and chlorophyll levels as both of poynomial and linear regression. stomatal conductance had a greater influence on plant height than the values of chlorophyll did. The effect of stomatal conductance on fruit formation and maturation period had an important relationships with the plant height. It has been shown that this influence had more impact than the chlorophyll values did.

Conclusion

It is crucial to detect accurately plant water sentitivity period and their water tolerant period . The information obtained from the previous researches show that determining the above-mentioned periods for many plants is important. As in drought stress, salinity stress is also caused the low yield by the approximately same mechanism in plant. In areas where water is scarce, the use of saline water for irrigation could cause serious problems in plant physiology. The previous findings indicated that drought-salinity stress could cause the similar physiological responses in their sensitive growth period.

It is important to know the pysiological response of plant during periods of stress in order to estimate the yield. The findings from this study indicates that the most sensitive period to salinity is ginofor formation period among the blooming, ginofor formation and fruit formation periods. Increased salinity stress caused a decrease in evapotranspiration. It was determined that the stomatal conductance significantly reduced depending on the salinity and chlorophyll content. The yield on the changes in chlorophyll content rather than changes in stomatal conductance plays a more important role. As the plant get older, impact of changes in stomatal conductance yield became insignificant. However, the chlorophyll content was determined to be more significant impact on the plant's final stage.

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INVESTIGATION OF THE EFFECTS ON GRAIN YIELD AND QUALITY OF PHYSIOLOGICAL CHARACTERISTICS IN BREAD WHEAT

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Abstract

This study was conducted at rained conditions in Diyarbakır during 2013-2014 grown seasons. The trial was conducted as a randomized complete block design with four replications. In the study, physiological parameters were investigated to determine their effect on the grain yield and quality parameters. 20 bread wheat advanced line originated from CIMMYT and 5 cultivars were used in this study. Genotypes was evaluated in terms of wet gluten value and sedimentation, protein content, test weight(TW), thousand grain weight(TGW), canopy temperature, chlorophyll content (SPAD), leaf area index, normalized vejatasyon index (NDVI), heading day, grain yield. As a result of analysis of variance, significant differences were determined in terms of TW, SPAD, heading day at level of 1% and 5%. Correlation analyses showed negative correlation between grain yield and canopy temperature, SPAD, protein, sedimentation value, wet gluten content; whereas positive correlation between grain yield and TGW, TW, NDVI.

As a result of this study; G7, G21, G22 and G24 had better performance in terms of TW, G11 and G16 for heading time, G1, G17, G19 and Pehlivan for chlorophyll content respectively. Because of heat stress early types of the genotypes must be used the breeding programs. Also, relationships between grain yield and physiological parameters should be investigated as comprehensively for breeding programs.

Keywords: Wheat, physiology, grain yield, quality.

Introduction

Wheat is an important crop being a staple food in Turkey (Yildirim et al., 2008). In Turkey, per person wheat consumption is 228.7 kg. Wheat production is 20.1 million tons under cultivated areas in Turkey (TSI, 2012). High grain yield in wheat has been the main aim in wheat, breeding studies was focused on attempts to improve the yield potential by new genotypes (Kusaksiz & Dere, 2010). Breeding programs are traditionally empirical, that is, the selection is based on the yield per se as a final integrator of all the processes occurring during the life of the plant. Selection efficiency could be increased if specific physiological and/or morphological attributes related to yield under specific environments could be identified and used as selection criteria for complementing traditional plant breeding (Acevedo, 1991). Total chlorophyll content per leaf area may be evaluated in a fast, single and nondestructive way using a portable chlorophyll meter like SPAD-502 (Soil-Plant Analysis Development Section, Minolta Camera Co., Ltd., Osaka, Japan). SPAD indirectly measures leaf chlorophyll and nitrogen (N) content. The linear or non-linear relationship between real leaf chlorophyll content and chlorophyll meter (SPAD) readings were reported in more than twenty published studies for different plants (Mulholland et al., 1997; Yamamoto et al., 2002; Uddling et al., 2007). Bread wheat (Triticum aestivum) is an important crop in Southeastern Anatolia of Turkey, where high temperatures and water stress often reduce plant growth and crop yields. Therefore, wheat yield is lowered. The ability of a cultivar to produce high and satisfactory yield over a wide range of stress and non-stress

environments is very important (Rashid *at al*, 2003). Some morphologic, physiologic or ecologic factors such as irradiance, leaf water status and measurement time (i.e., morning vs. afternoon), crop growth stage, heat stress, mineral status of the soil and irrigation may affect SPAD readings. Also year, location and cultivar differences may have an effect on leaf greenness and SPAD readings (Bavec and Bavec, 2001; Rharrabti et al., 2001).

The aim of this study was to determine of the effects on grain yield and quality of physiological characteristics in bread wheat in Turkey and to evaluate whether the physiological characteristics could be used as selection criteria to obtain stable and bread wheat genotypes.

Materials and methods

The experiment is conducted in randomized complete block design with four replications in GAP International Agriculture research and Traning Center field. Studies were conducted under the rainfall condition with 20 advenced bread wheat lines and 5 cultivarsb(Table-1). Each of the five commercial wheat varieties and a 20 adventes lines were grown in 6 rows where each row length was kept 5 meter by maintaining a row to row distance of 20 cm. A seedling rate of 450 seed/m^2 was used in each planting. The trials were managed with optimum nutrient application (60 N: 60 F: 0 K) while the weeds were controlled with of herbicides. The data was statistically computed by JMP 5.0.1 program as well as for correlation.

Results and discussion

Table-1. Grain yield and quality results of the trial carried out during 2013/2014 seasons

genotypes	**H.Time**		**SPAD(Heading time)**		**TW(kg l^{-1})**		**Grain Yield(kg.ha^{-1})**
1	112.0	gh	48.2	ad	77.1	dh	2825
2	113.5	eg	46.6	af	77.2	ch	3110
3	112.5	fh	47.2	af	77.0	eh	3347
4	112.5	fh	44.9	bg	73.6	gh	2566
Dinç	111.0	hı	43.8	fh	77.3	ch	3859
6	111.5	hı	41.0	h	79.5	af	3358
7	112.5	fh	46.6	af	83.1	ab	3257
8	116.5	bc	45.8	ag	81.5	af	3502
9	114.5	de	46.8	af	81.0	af	3193
Pehlivan	118.0	ab	49.0	a	81.5	af	2593
11	107.0	j	45.4	ag	80.9	af	2830
12	110.0	ı	42.2	gh	77.8	bg	2835
13	112.0	gh	47.4	af	72.5	h	2617
14	111.5	hı	45.1	bg	73.3	gh	3195
Cemre	119.0	a	44.9	bg	79.6	af	2897
16	107.0	j	46.5	af	79.8	af	2870
17	113.5	eg	48.4	ac	79.6	af	3340
18	113.5	eg	44.4	dh	76.7	fh	3206
19	111.0	hı	48.7	ab	76.7	fh	2854
Sagitario	115.5	cd	48.0	ae	78.5	bg	2925
21	112.0	gh	44.2	eh	83.9	a	3915
22	114.5	de	42.1	gh	82.4	ac	2996
23	112.0	gh	46.1	af	76.3	fh	2873
24	111.0	hı	45.3	ag	82.3	ad	2785
Adana-99	114.0	df	44.8	ch	82.0	ae	2694
LSD(0.05) :	1.8**		3.8*		5.2**		N.I
CV(%):	0.8		4.1		3.2		14.9

, * Significant at **0.05** and **0.01** levels of probability, respectively. **N.I:**Not importend, **H.Time:**Heading time, **SPAD:**Chlorophyll content, **TW:** Test weight

As shown in Table-1, in 2013-2014 growing season, grain yield has been low due to the frost on March 31, temperature and drought stress. Also, significant differences were determined in terms of TW, SPAD, heading day at level of 1% and 5%

Table 2. The correlation between the parameters

Features	G.Yield	H.Time	C.Temp.	SPAD	YAI	NDVI	BD	TW	PRT	SDM
H.Time	-.139									
C.Temp.	-.384**	-.096								
SPAD	-.406**	.127	.086							
YAI	.002	.285*	-.211	-.096						
NDVI	.321*	.458**	-.294*	-.013	.108					
TGW	.440**	-.025	-.440**	-.298*	.269	.162				
TW	.596**	.130	-.251	-.174	.153	.291*	.627**			
PRT	-.757**	.097	.195	.500**	.004	-.176	-.581**	-.702**		
SDM	-.742**	.120	.201	.516**	.005	-.166	-.574**	-.663**	.996**	
W.GLT	-.756**	.099	.194	.498**	.006	-.175	-.578**	-.697**	.999**	.996**

C.Temp.= Canopy temperature, **YAI**: Leaf area index, **NDVI**: Normalized vegetation index, **TGW**: Thousand grain weight, **PRT**: Protein, **SDM**: Sedimentation, **W.GLT**: Wet gluten, **G.Yield**: Grain yield,

Correlation analyses showed negative corelation between grain yield and canopy temprature, SPAD, protein, sedimantation value, wet gluten content; whereas pozitive corelation between grain yield and TGW, TW, NDVI. Several studies, Although a positive correlation was determined beetwen grain yield with SPAD(Yildirim et al 2009). According to results of this study, a negative correlation was determined beetwen grain yield with SPAD. This situation, It is believed to be caused by frost. Because, genotype is delayed 15-20 days as the vegetation.

Conclusion

Grain yield has been low due to the frost on March 31, temperature and drought stress. As a result of this study; G7, G21, G22 and G24 had better performance in terms of TW, G11 and G16 for heading time, G1, G17, G19 and Pehlivan for chlorophyll content respectlively. These genotypes must be used the breeding programs. Also, Because of escape than the heat stress, early heading genotypes(G11 and G16) should be evaluated. Relationships between physiological parameters and grain yield, quality should be investigated as comprehensively for breeding programs.

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COMPARISION OF REGISTERED FLAXSEED CULTIVARS IN TERMS OF MINERAL AND NUTRITIONAL COMPOSITION AND HARVES EFFICIENCY

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Abstract

Flaxseed is a valuable product, containing 34-35% fat, 23% protein, 22% nitrogen-free extract, 9% fiber, 3% ash and 8% water. It is widely used in chemical, food and pharmaceutical industry. Health promoting activity of flaxseed oil is mainly connected with the presence of essential fatty acids (EFA). On average, it consist of 50–58% α-linolenic acid (ω3), 15% linoleic (ω6) and 17% oleic acid (ω9) glycerides. However, the beneficial for health ω3/ω6 ratio may vary depending on the cultivar type. To achieve both the high yields and the highest possible quality of raw material, new flax cultivars are introduced to agricultural production, as genetic factor can significantly affect flax ontogeny, including fat content and composition.

The research aimed at selection of flax cultivars best for the production of the highest quality oil (the most desired set of EFA), as well as the cultivars whose seeds accumulate the lowest number of nutritional values. Namely, 28 flax cultivars were subjected to analysis, all registered in such countries as France, Netherlands, Austria, UK, Estonia, Canada, Romania, Czech Republic, Germany, Belgium and Poland. The analysis included comparison of such parameters as: crop yield, calorific value of flax seed, content of mineral and organic components, fat and protein yields, and also fatty acids chemical composition. The results showed that for different cultivars the crop yield may vary from 1.09 to 2.88Mg·ha^{-1} with corresponding calorific values from 121 to 325 MJ·ha^{-1}. At the same time fat and protein yield averages respectively 420-1162kg·ha^{-1} and 302-764 kg·ha^{-1}.

Key words: *flaxseed, mineral and organic composition, mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), saturated fatty acids (SFA)*

Introduction

Oil flax is a raw material used in different industries, including chemical, pharmaceutical, food, fodder, paper, textile as well as medicine. Its seeds, in comparison to other oil plants, are the most effective, stabile and cheap source of linoleic acid from *n-3* family (Rumińska 1990, Tokarz 1994). The obtained esters of linseed oil, produced from cold – pressed oil, can be used for supplementation of different food products which are important in diet – related diseases in humans (Jelińska et al. 2003, Obiedzińska and Waszkiewicz-Robak 2012).

The application of 10% addition of ground brown and yellow flax seeds to wheat or mixed wheat and rye bread baking, results in significant increase in total protein, two – fold increase in dietary fibers and three – fold growth of crude fat content as compared to standard bread. Similar tendency, namely the considerable increase, was recorded with regard to Ca content– by 70%, Cu – by 150% and Mg – up to 200%. Five – fold increase in the quantity of linolenic acid was reported in fatty acids profile of bread Jelińska et al. (2003). Usually, the

content of this acid in flax seeds amounts from 52 to 58 % of total fatty acids (Ajuyah et al. 1991). The presence of polyunsaturated fatty acids influences decreased risk of the occurrence of cancer and the bread with the addition of linseed flouris recommended in glutene – free diet (Gambuś et al. 2009). Linseed oil is rich in indispensible polyunsaturated fatty acids (PUFA), overrunning, in this respect, all more important oil plants, as well as fish fat (Jelińska et al. 2003, Zając et al. 2010).

The fact that flax seeds are rich in fibre, slime, phenolic compounds, as well as indispensible unsaturated fatty acids, mainly from *n-3* family. From these reasons they are used in animal feeding.The basic aim of flax seeds or linseed oil application in animal feeding dose is the increase in polysaturated fatty acids (PUFA) content in animal products, as well as the decrease in mutual ratio of fatty acids from *n-6* and *n-3* families, which should reach a ratio of 4-5:1. Higher amount of PUFA from family in the human diet reduces the overall cholesterol level in blood, and through the increase in HDL lipoproteins concentration decrease LDL lipoprotein concentration in blood serum (Tuberoso et al. 2007).

Full meeting of the increasing demands for linseed oil production for consumption purposes can be obtained through the increase in oil flax and fiber flax cultivation area, accompanied by increased yielding and appropriate choice of flax cultivars. At the same time, reduction in flax seed originating from cultivation of fiber flax as a byproduct should be introduced Zając (2004). The level of flax yielding in the different countries is low, especially as compared to basic crops, as well as highly variable according to the years (Diepenbrock et al. 1995, Arslan et al. 2011). This fact points to the need of a proper selection of flax cultivars, as well as the answer to the question if agro –meteorological conditions significantly effect on genetically determined composition of fatty acids. The aim of this work was a comparison of available, registered flax cultivars and selection of flax cultivars best for the production of the highest quality oil, as well as the cultivars whose seeds accumulate the lowest number of nutritional values.

Materials and methods

In the years 2009-2011, in Experimental Station belonging to the Department of Plant Cultivation in Pawłowice in Poland (eastern longitude 17°12´ and northern latitude 51°31) the field experiment was conducted, according to the method of subblocks in correlated system, in four replications. The research involved 30 cultivars of oil and fiber flax and old varieties originating from different countries. Winter wheat was a forecrop for the oilseed flax. The pre-sowing fertilization involved: 46% triple superphosphate, in the amount of 40 kg $P_2O_5 \cdot ha^{-1}$, 60% potassium salt, in the amount of 70 kg $K_2O \cdot ha^{-1}$ and 46% urea - 20 kg $N \cdot ha^{-1}$ .Thirty four coma four percent ammonium nitrate was introduced in a growth stage of flax plant (so – called herring – bone phase), in the amount of 20 kg $N \cdot ha^{-1}$, in the form of top – dressing fertilization.

The soil in Pawłowice is classified as autogenic, brown soil, originating from light loam on medium loam, assigned to agriculturally usable good wheat complex, of soil class III b. In the years of investigation the phosphorus content in the soil ranged from a medium to a very high level, potassium content was between a low and a very high range, magnesium – high to very high content, while pH range was slightly acidic.

The Experimental Station is situated in the hottest region of Lower Silesia, which is the hottest region of Poland. The climate in the region of Wrocław possesses the features typical for tepid climate of the temperate zone. In order to characterize in the experimental years the weather conditions during the vegetation period of the flax, the indicator developed by Sielianinov was used (Molga 1986, Radomski 1987). According to Sielianinov, during the summer months, when there is the greatest water demand by plants, the sum of the temperatures divided by 10 gives the approximate numerical result of evaporation sum, resulting from the measurement with Wild's balance-evaporimeter. On this basis, Sielianinov developed an index, which he called the coefficient of water security or the conventional moisture balance (hydrothermic coefficient): $K = 10P / \Sigma t$.

In this work, in order to determine the moisture conditions during the growing season of plants, eleven different ranges were taken adopted: for Sielianinov's hydrothermic coefficient K below 0.3 - extremely dry; between 0.31 and 0.5 - very dry; 0.51 ÷ 0.7 – dry; 0 71 ÷ 0.9 - quite dry; 0.91 ÷ 1.0 - moderately dry; 1.01 ÷ 1.2 – average; from 1.21 to 1.5 – optimal; 1.51 ÷ 1.9 - quite humid; 1.91 ÷ 2.4 – humid; 2.41 to 3.0 - very humid; and above 3.1 -extremely humid. Moreover, the agroclimatic conditions were described using indexes proposed by Kaczorowska (1962).

Precipitation was expressed as percentage of long-term average of each month, assuming the scale: less than 25 - a period of extremely dry; 26-49 - very dry; 50-74 – dry; 75-124 – normal; 125-149 - humid, 150-174 - very humid; and above 175 – extremely humid period.

The temperature course during the experimental period was evaluated by their deviation from the mean value for the multi-year period. Expressed in degrees it was: above 2.0 - very warm period; from 2.0 to 0.6 – worm; between + 0.5 and - 0.5 – normal period; from - 0.6 to - 2.0 – cold; and below - 2.0 - very cool period.

The course of weather conditions in the years of investigation, *i.e.* distribution of the temperature and rainfall were presented in table 1.

Table 1

Meteorological conditions during growth periods in the years 2010-2012

Month	T_{mean} (ºC)	$D(T_{mean})$* (ºC)			Σ_{prec} (mm)	$\Sigma_{rainfall}$ (mm) $\%\Sigma_{rainfall}$ (%)			Sielianinov coefficient K*		
		2010	2011	2012		2010	2011	2012	2010	2011	2012
III	3.4	0.8 (wp)	1.0 (wp)	1.2 (wp)	35	44,9 (130%)	45,2 (131%)	48,3 (140%)	2.9 (vw)	3.2 (ew)	3.4 (ew)
IV	8.7	0.6 (wp)	3.2 (vwp)	3.3 (vwp)	35	45,4 (128%)	27,0 (76%)	30,9 (87%)	1.6 (qw)	0.8 (qd)	0.9 (ds)
V	14.2	-1.5 (cp)	0.6 (wp)	0.0 (n)	53	140,7 (264%)	49,4 (93%)	67,6 (127%)	3.6 (ew)	1.1 (a)	1.5 (o)
VI	17.1	0.8 (wp)	2.1 (vwp)	-1.3 (cp)	67	32,9 (49%)	95,7 (143%)	141,7 (211%)	0.6 (d)	1.7 (qw)	3.0 (vw)
VII	18.9	2.5 (vwp)	-0.7 (cp)	0.7 (wp)	86	78,6 (91%)	170,9 (199%)	134,2 (156%)	1.2 (a)	3.0 (vw)	2.2 (w)
VIII	18.4	0.6 (wp)	0.9 (wp)	0.9 (wp)	70	109,1 (157%)	78,9 (113%)	53,5 (77%)	1.9 (qw)	1.3 (o)	0.9 (qd)
IX	13.8	-1.3 (cp)	1.7 (wp)	1.5 (wp)	47	134,1 (282%)	30,4 (64%)	12,0 (25%)	3.6 (ew)	0.7 (d)	0.3 (ed)
mean/ sum	13.5	0.3 normal	1.2 warm	0.9 warm	393	585,7 (149%)	497,5 (126%)	488,2 (124%)	2.2 (w)	1.7 (qw)	1.7 (qw)

Abbreviations: T_{mean} - Multiyear mean temperature (years 1980-2009); $D(T_{mean})$ - Deviation from multiyear mean temperature; Σ_{prec} - Sum of multiyear precipitation (for the years 1980-2009); $\Sigma_{rainfall}$ - Sums of rainfall (mm) in the months of the vegetation period of 2010-2012; $\%\Sigma_{rainfall}$ - percentage of multiyear precipitation sum.
* legend: (vwp)- very warm period, (wp) -warm period, (n)- normal, (cp)- cold period, (vcp)- very cold period; (ed) - exremely dry (drought), (vd) - very dry(drought), (d) - dry (dry weather), (qd) - quite dry(dry weather), (md) - medium dry (dry weather) , (a) - average, (o) – optimal, (qw) - quite wet, (w) – wet, (vw) - very wet, (ew) - extremely wet.

Fatty acids profile was determined using gas chromatograph equipped with the flame ionization detector (FID) and capillary column ZB-WAX (30 m × 0,25 mm i.d., 0,25 µm film thickness). Helium with a velocity flow-rate of 20 m s^{-1} was used as a carrier gas, the temperature of injector was 250°C, and the detector temperature was equal 270°C. The column oven was working under the programmable temperature regime: 80°C maintained for 1 min, first temperature ramp to 140°C at the rate of 20°C min^{-1}, than rise to 200°C at the rate of 10°C min^{-1}, and further rise to 250 °C at the speed of 30°C min^{-1}. The maximum temperature of 250°C was kept for 6 min. Total time of analysis was 18 min. Qualitative and quantitative analysis of chromatograms was performed on the basis of comparison respectively the retention times and the peaks areas of fatty acids methyl esters in the examined sample with the methyl esters standards (Supelco 37 Component FAME Mix).

Results and discussion

Oil flax shows very high variability of yield in which mainly depends on the course of metheorological conditions, as well as environmental conditions. The research conducted by a number of authors proved considerable fluctuation of yield size data according to the years, ranging from 0.31 t/ha to over 3.0 $t \cdot ha^{-1}$, while within a single year the difference between maximum yield could reach a value of 1.95 $t \cdot ha^{-1}$ (Diepenbrock et al. 1995).

In this study, no significant differences in flax fielding, resulting from the course of weather conditions, was obtained, and the mean value of seed yield was equal 2.3$t \cdot ha^{-1}$ (tab. 2). However, significant differences in yielding between particular cultivars were observed.

Table 2

Content of dry matter, organic and mineral nutrients, energy value in seeds of oil flax (*Linum usitatissimum* L.) and the yield of crude fat and total protein and energetic value of grain yield.

Specification	Dry matter [g·kg^{-1}]	Total protein [g·kg^{-1}]	Crude fat [g·kg^{-1}]	Crude fibre [g·kg^{-1}]	Crude ash [g·kg^{-1}]	N, *Nitrogen* [g·kg^{-1}]	Mg, *Magnesium* [mg·kg^{-1}]	P, *Phosporus* [mg·kg^{-1}]	K, *Potassium* [mg·kg^{-1}]	Ca, *Calcium* [mg·kg^{-1}]	Na, *Sodium* [mg·kg^{-1}]	Energy value [MJ ha^{-1}]	Yield of seeds [Mg·ha^{-1}]	Yield of crude fat [kg·ha^{-1}]	Yield of total protein[kg·ha^{-1}]	Energy value [MJ ha^{-1}]
Years																
2010	941	269	374	71.3	44.4	43.0	423	582	566	509	30.7	10.9	2.35	875	631	256
2011	945	264	384	74.9	41.8	42.3	387	473	432	120	25.3	11.1	2.31	886	609	257
2012	940	268	379	74.6	41.2	44.8	392	545	566	120	37.3	11.0	2.24	847	600	247
$LSD_{0,05}$	***	NS	*	NS	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	NS
Cultivar																
Agatha	940	281	366	79.9	41.4	45.0	407	550	567	107	36.7	10.8	2.13	779	602	230
Antares	939	275	387	73.0	38.0	43.9	400	517	633	123	33.3	11.1	1.09	421	303	121
Athlanta	943	257	380	79.1	39.0	41.2	417	513	477	123	36.7	10.9	2.67	1014	685	290
Barbara	944	266	385	80.1	37.7	42.6	367	557	497	110	26.7	11.0	2.00	764	554	221
Dakota	944	267	368	85.1	38.2	42.8	367	543	497	107	23.3	10.8	2.36	869	630	255
Electra	942	295	361	74.6	39.5	47.2	423	530	477	113	33.3	10.8	2.57	927	758	277
Flanders	945	257	397	75.5	37.5	41.1	360	517	540	110	30.0	11.2	2.48	984	641	278
Floriana	946	242	404	79.0	39.0	38.7	353	497	570	110	33.3	11.1	2.88	1162	696	320
Florinda	937	269	373	60.1	47.7	43.0	400	537	390	123	26.7	10.7	2.63	978	708	280
Hermes	943	269	375	72.2	53.9	43.1	403	510	563	107	30.0	10.7	2.62	981	702	279
Jantarol	937	273	375	66.3	41.7	43.6	413	533	380	117	23.3	10.6	2.09	785	572	223
Jitka	941	291	360	78.6	42.4	46.5	353	553	513	113	43.3	10.5	1.96	701	566	205
Landracea	944	260	388	71.9	38.6	41.7	417	557	513	110	30.0	10.9	2.35	908	610	255
Linda	942	261	376	86.8	44.5	41.8	367	490	470	110	43.3	10.7	2.66	1002	692	285
Lirina	946	252	403	71.6	38.9	40.2	417	517	487	109	33.3	11.2	2.23	898	560	249
Marylin	936	271	367	65.2	54.8	63.3	397	520	467	120	26.7	10.5	2.43	891	659	254
Mc Gregor	942	257	387	74.3	39.2	41.1	433	550	513	123	23.3	10.9	1.64	635	423	179
Micael	946	249	396	76.8	37.6	39.8	397	523	513	127	36.7	11.0	2.21	876	549	243
Midin	945	252	397	76.1	38.4	40.2	423	547	623	117	30.0	11.2	2.45	970	616	275
Norman	944	271	386	78.0	40.0	43.4	427	533	630	113	43.3	11.1	1.63	630	444	182
Olin	948	251	402	70.7	36.8	40.1	400	533	550	117	33.3	11.3	2.24	900	563	254
Oliwin	937	277	363	64.1	48.8	44.3	423	527	490	130	26.7	10.7	2.51	910	699	268
Opal	937	272	372	61.4	44.4	44.0	400	527	440	127	26.7	10.8	2.68	996	731	289
Pluin	941	239	406	76.6	39.7	38.3	447	537	717	123	40.0	11.4	2.11	855	504	239
Raulinus	946	255	389	81.7	38.8	40.8	430	550	530	127	26.7	11.6	2.68	1042	681	310
Royal	944	263	384	65.5	51.3	42.1	340	537	507	117	26.7	11.7	2.48	956	652	291
Szafir	937	280	354	58.0	67.5	44.8	403	550	397	130	23.3	11.4	2.08	735	580	237
Venica	935	286	351	77.2	40.6	45.5	437	547	530	120	30.0	12.2	2.67	937	764	325
Viking	943	281	364	73.5	37.4	45.0	387	533	543	120	26.7	11.2	2.30	839	646	258
Viola	943	289	356	75.0	41.3	46.3	417	563	617	110	30.0	11.1	2.10	741	607	233
$LSD_{0,05}$	***	**	***	*	NS	NS	NS	***	***	NS	NS	*	**	**	**	**

$LSD_{0,05}$, NS- nonsignificant; * Significant at the 0,05 level of probability; ** Significant at the 0,01 level of probability; *** Significant at the 0,001 level of probability

In the three – year experiment, the highest – yielding cultivar proved to be Floriana, the Romanian fiber cultivar featuring the yield of 2.88 $t \cdot ha^{-1}$, while the poorest yield size characterized Opal, the oil cultivar whose yield amounted 2.68 $t \cdot ha^{-1}$. The differences in cultivars yielding were also reported in the research Mohammadi et al. (2010). The author proved that the course of the weather conditions did not affect the percentage of protein, ash, fiber, N, P, K, Mg and Ca contents in flax seeds, and also the yield of fat, protein and energy efficiency from the area unit. In the three – year experiment, climatic conditions did significantly influence on percentage of fat content in seeds and their average values were equal respectively 37.9% and 30 $mg \cdot 1000\ g^{-1}$.

Significant differences between particular cultivars were recorded for the content of fat, protein, fiber, P and K in flax seeds and for the first two of the difference ranged approximately 5.5%. The mean content of fat and protein in seeds equalled respectively 37.9% and 26.7%, which corresponds with the results reported by Diederichsen, Raney (2010) and Laza, Pop (2012). A genetic factor did not affect the content of Mg, Ca and Na, which was also confirmed in the research by Laza and Pop (2012). Different findings were reported by Kiralan et al. (20109, who indicated significant effect of a cultivar factor on the content of mineral components Ca, Mg, Na, K, P at simultaneous considerable differentiation of some their values.

The yield size and the content of organic components in the seeds correspond with the yield of protein (Dordas 2011), fat (Khourang et al. 2012) and energy efficiency from 1 hectare. Mean yield of fat in the experiment was about 870 kg from 1ha. As far as the field of fat was concerned, the best cultivars proved to be: Floriana, Raulinus, Athlanta, Linda and Opal and the results obtained for these cultivars were higher from the mean ones by 290, 172, 144, 132 and 126 $kg \cdot ha^{-1}$ respectively. Considering the yield size of protein, the highest values characterized flax cultivars: Venica – 764.3kg, Electra – 758.4kg, Opal- 730,1kg, Florinda- 708.0kg and Hermes – 702.4kg. The most energy – efficient were cultivars: Venica, Floriana, Raulinus, Royal and Athlanta, with field size values ranging from 325.4 to 288.9 MJ, at mean value for the experiment amounting 253.6 MJ z 1 ha. Energetic value in the experiment by Poiša and Adamovičs (2011) obtained the level higher by nearly 30%.

Warm year 2012 affected on the content of the following acids $C_{8:0}$, $C_{14:1}$, $C_{18:1n9c}$, $C_{22:1n9}$, $C_{24:1}$, MUFA and OFA. Similar findings were reported in the research of Gambuś et al.(2009). The year 2010, with the lowest 24 – hour – temperature and the highest precipitation during flax growing period, favored higher contents of $C_{14:0}$, $C_{18:0}$ and $C_{22:6n3}$. On the other hand, the year 2011, described according to Sielianinov coefficient as quite wet, featuring the highest mean air temperature during plants growing period, provided for higher contents of $C_{18:3n3}$, $C_{20:2}$, $C_{20:3n6}$, $C_{22:2}$, $C_{24:0}$ and PUFA. Different results were obtained by Zając et al. (2001), who proved that only the content of $C_{14:0}$ was modified by agro-meteorological factors.

Many authors, regardless of the investigated factors, reported the differences in fatty acids composition in flax cultivars (Herchi et al. 2011, Klimek-Kopyra et al. 2012). Usually, the analysis includes the use of the mutual proportions and the relations of particular acids or their groups, like: MUFA, PUFA, SFA, UFA, DFA, OFA, ∑P.M.L ODR, LDR, as well as COX coefficient (Zając et al. 2001, Piłat, Zadernowski 2010, Herchi et al. 2015, Maknatjou et al 2015).

The highest relations between PUFA and MUFA took place in 2011 and amounted 1:2.77. No significant differences in the proportion of PUFA/MUFA and of UFA/SFA in cultivar were found, although these values fluctuated from 2.23 to 3.12 for PUFA/MUFA and from 4.96 to 6.15 for UFA/SFA. According to the research by Zając et al. (2001), the years and cultivars did not influence on MUFA, PUFA, SFA and UFA, as well as, there mutual proportions, nevertheless, the value of obtained were comparable.

Table 3

Selected fatty acids content of oil flax seeds (*Linum usitatissimum* L.), % of total fatty acid.

Specification	<C16	Palmitic acid (16:0)	Palmitoleic (C16:1)	Stearic ac. (C18:0)	Oleic acid (C18:1n9)	Linoleic (C18:2n6)	Linolenic (C18:3n3)	Linolenic (C18:3n6)	Arachidate (C20:0)	Unsaturated C20	Behenic acid (C22:0)	Unsaturated C22	C24:0 + C24:1	SFA	MUFA	PUFA[1)]	DFA[2)]	OFA[3)]	COX value[4)]	ODR[5)]	LDR[6)]	Athero[7)]
Years																						
2010	0.55	8.79	0.14	4.83	21.7	14.0	46.3	0.14	0.32	0.26	0.12	0.98	0.97	15.5	23.2	61.3	89.4	8.88	11.5	72.9	77.1	9.05
2011	0.50	8.60	0.17	4.39	21.3	13.5	46.7	0.15	0.37	0.22	0.13	0.51	2.17	15.5	23.0	61.5	88.9	8.70	11.5	72.3	77.0	9.17
2012	0.61	9.12	0.15	4.35	24.3	13.6	44.0	0.14	0.30	0.29	0.10	0.99	1.29	15.4	26.1	58.5	89.0	9.22	11.6	72.4	77.0	8.61
$LSD_{0,05}$	NS	*	NS	**	***	NS	**	NS	NS	NS	NS	**	NS	NS	***	***	NS	*	NS	NS	NS	*
Cultivar																						
Agatha	0.66	8.78	0.15	4.07	20.1	15.1	46.3	0.14	0.38	0.75	0.10	0.92	1.91	15.1	22.4	62.5	89.0	8.88	11.2	72.0	76.1	9.24
Antares	0.61	8.70	0.15	4.56	23.3	13.1	44.8	0.17	0.30	1.12	0.16	0.79	1.82	15.3	25.7	59.0	89.3	8.79	12.0	75.1	76.3	8.41
Athlanta	0.58	9.22	0.19	4.81	21.9	13.3	46.1	0.16	0.41	0.90	0.08	0.99	0.95	16.0	23.4	60.6	88.8	9.32	11.1	70.2	76.8	9.21
Barbara	0.57	9.15	0.19	5.07	23.2	12.2	45.3	0.13	0.36	1.02	0.14	0.95	1.34	16.2	24.9	58.8	88.8	9.25	12.5	75.8	78.3	7.59
Dakota	0.49	8.96	0.15	4.29	23.6	13.5	45.6	0.11	0.30	0.82	0.11	0.70	0.90	15.0	25.0	60.0	89.3	9.05	11.1	70.7	76.9	8.75
Electra	0.50	8.35	0.16	4.94	22.0	14.0	44.1	0.12	0.38	0.99	0.09	0.89	2.99	16.5	24.1	59.4	88.4	8.45	11.3	70.7	77.7	8.93
Flanders	0.54	8.50	0.15	4.75	23.5	13.9	45.0	0.12	0.32	0.82	0.10	0.69	1.32	15.2	25.0	59.8	89.6	8.59	11.7	73.4	76.9	8.62
Floriana	0.55	9.10	0.14	4.38	20.1	14.2	48.5	0.14	0.33	0.67	0.09	0.77	0.63	15.2	21.1	63.6	89.1	9.18	11.0	70.1	77.7	8.35
Florinda	0.53	9.57	0.18	4.49	23.3	13.3	43.8	0.16	0.28	0.81	0.12	0.87	2.21	16.6	25.1	58.3	87.9	9.68	11.4	71.4	77.2	9.02
Hermes	0.50	8.85	0.13	3.86	20.8	14.1	48.1	0.11	0.37	0.55	0.16	0.54	1.56	14.8	22.3	62.9	89.1	8.95	11.3	72.1	76.8	9.49
Jantarol	0.58	8.67	0.15	4.47	24.5	12.9	44.6	0.12	0.28	0.86	0.15	0.70	1.63	15.1	26.4	58.5	89.3	8.77	11.3	69.8	79.0	9.39
Jitka	0.88	8.13	0.20	4.57	24.0	12.9	44.4	0.27	0.40	0.83	0.09	1.24	1.52	15.1	26.3	58.6	89.5	8.24	11.0	70.2	76.8	9.73
Landracea	0.45	9.56	0.20	3.87	21.8	13.7	44.5	0.16	0.20	0.67	0.13	0.81	3.49	16.5	24.2	59.4	87.4	9.66	11.6	72.9	76.9	9.09
Linda	0.56	9.24	0.14	4.18	24.6	13.0	44.3	0.17	0.30	0.76	0.10	1.03	1.20	15.3	26.2	58.5	88.9	9.33	12.2	75.7	79.4	8.72
Lirina	0.48	9.06	0.14	4.47	20.3	13.7	48.8	0.12	0.34	0.58	0.14	0.90	0.60	15.1	21.5	63.4	89.3	9.14	11.3	70.0	77.1	9.08
Marylin	0.51	8.80	0.15	4.24	23.3	13.7	44.8	0.16	0.36	0.90	0.11	0.79	1.81	15.1	25.2	59.7	89.1	8.89	11.5	73.0	77.0	8.96
Mc Gregor	0.42	9.50	0.15	4.40	20.9	15.4	44.9	0.15	0.29	0.70	0.13	0.71	1.92	15.9	22.7	61.4	88.5	9.60	11.7	74.3	77.9	8.42
Micael	0.66	8.88	0.22	5.26	22.3	13.7	44.4	0.15	0.53	1.22	0.06	0.90	1.15	16.5	23.8	59.7	88.7	8.97	11.2	70.8	75.2	9.25
Midin	0.53	9.26	0.10	4.62	21.9	13.2	47.3	0.11	0.28	0.61	0.13	0.75	0.74	15.6	23.2	61.3	89.1	9.36	11.8	74.8	76.2	8.79
Norman	0.58	9.27	0.14	4.76	22.6	13.7	45.7	0.12	0.36	0.59	0.14	0.68	1.01	16.0	23.9	60.1	88.8	9.37	11.7	73.9	76.2	8.74
Olin	0.52	9.01	0.14	4.48	22.9	13.8	44.2	0.14	0.34	0.95	0.12	0.94	2.09	15.9	24.9	59.2	88.6	9.10	11.2	70.0	76.5	9.15
Oliwin	0.52	8.01	0.16	3.44	20.7	14.0	49.8	0.13	0.22	0.79	0.07	0.53	1.08	12.9	22.3	64.8	90.5	8.10	12.0	75.9	76.9	9.06
Opal	0.65	7.50	0.17	4.95	23.7	13.5	45.0	0.16	0.46	1.06	0.09	0.74	1.50	14.9	25.3	59.9	90.1	7.58	11.7	73.8	77.0	8.70
Pluin	0.52	9.82	0.17	5.34	23.7	13.5	43.5	0.15	0.30	0.85	0.12	1.02	0.61	16.8	24.9	58.3	88.5	9.93	11.3	70.5	76.5	9.29
Raulinus	0.59	8.55	0.12	5.35	23.0	12.7	45.3	0.13	0.30	0.76	0.19	1.15	1.45	15.9	25.0	59.1	89.4	8.64	11.8	73.8	78.0	9.60
Royal	0.55	9.12	0.16	4.35	21.4	13.7	47.1	0.12	0.29	0.68	0.13	0.79	1.28	15.1	23.2	61.7	89.3	9.21	11.6	74.0	78.7	8.83
Szafir	0.63	8.31	0.12	4.57	22.5	13.1	46.7	0.13	0.31	0.78	0.16	0.81	1.44	14.9	24.3	60.8	89.7	8.39	11.0	69.7	76.1	9.17
Venica	0.55	8.65	0.13	4.57	23.2	14.0	45.9	0.10	0.30	0.80	0.09	0.70	0.77	15.1	24.2	60.8	89.5	8.76	11.5	71.9	75.8	8.65
Viking	0.44	8.37	0.12	4.56	21.9	13.1	46.0	0.15	0.32	0.98	0.11	0.84	2.68	15.6	23.9	60.4	88.9	8.45	12.8	79.0	76.9	8.98
Viola	0.38	8.17	0.09	4.08	22.2	16.5	45.4	0.22	0.3	0.67	0.12	0.74	0.81	14.0	23.2	62.8	90.1	8.24	11.1	70.0	75.0	9.01
$LSD_{0,05}$	NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS

[1)](PUFA = Essential Fatty Acids - EFA); [2)] DFA – index of desirable fatty acids (sum of unsaturated fatty acids and stearic acid, i.e. neutral and hypocholesterolemic acids, (C18:0+UFA); [3)] OFA - sum hypocholesterolemic fatty acids (C l4:0 + C 16:0); [4)] COX (Calculated oxidizability value) = [1 (18: 1%) + 10.3 (18: 2%) + 21.6(18: 3%)]/100 ; [5)] ODR (Oleic desaturation ratio) = [% C18: 2 + % C18: 3 / % C18: 1 + % C18: 2 + % C18: 3] × 100; [6)] LDR ((Linoleic desaturation ratio) = [% C18: 3 / % C18: 2 + % C18: 3] × 100; [7)] Athero – sum of C12:0, C14:0 and 16:0 acids. The difference between sum of presented in table fatty acids and 100% reflect the sum of FA with uneven number of carbons in the molecule..

Conclusion

- The weather conditions did not modified the percentage of fat and protein content in flax seeds, as well as, they did not influence on the yield of fat, protein and energy efficiency from 1 ha.
- The obtained seed yield within the range from 1.09 to 2.88 $t \cdot ha^{-1}$, fat content 421.0÷1161.8 $kg \cdot ha^{-1}$and protein content – 302.5÷764.3 $kg \cdot ha^{-1}$resulted from the cultivar factor. Among the compered fibre cultivars, the highest seed yield were found out for Floriana, Venica, Hermes, Electra, Marylin, while in oil cultivars the highest yield values characterised Opal, Raulinus, Athlanta, Oliwin, Flanders.
- Genetic factor determined the content of $C_{16:0}$, $C_{18:0}$, $C_{18:2}$, and $C_{20:1}$ acids.
- The years of research significantly affected the fatty acids profile of flax oil, namely: $C_{8:0}$, $C_{14:0}$, $C_{14:1}$, $C_{18:0}$, $C_{18:1n9c}$, $C_{18:3n3}$, $C_{20:2}$ and $C_{20:3n6}$ (given as sum), unsaturated C_{22} acids (namely $C_{22:1n9}$, $C_{22:2}$, $C_{22:6n3}$), $C_{24:0}$, $C_{24:1}$, MUFA, PUFA, OFA and their mutual proportion PUFA/ MUFA.

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A COMPARATIVE STUDY OF HAIRY ROOT INDUCTION EFFICIENCY IN TWO MEDICINALLY IMPORTANT PLANTS USING AGROBACTERIUM RHIZOGENES

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Abstract

Medicinal plants have been used from ancient times to attempt cures for diseases and their importance has become increasingly apparent. Despite advancements in the chemical synthesis of secondary metabolites, extracted from plant sources is the only way to achieve many valuable secondary metabolites. Recent advances in plant genetics and recombinant DNA technology, contributed to improve and strengthen research on the biosynthesis of secondary metabolites. Hairy Root Culture is the new route for large scale secondary metabolite production because of their fast and plagiotropic growth, genetic and biochemical stability. Using recombinant DNA technology allowed to changes in gene expression that are involved in the biosynthesis of secondary metabolites. High potential of hairy roots to produce valuable metabolites and commercialization it's by using bioreactor system, have been attracted by private companies. Different explants of two medicinally important plants *Agastache foeniculum* and *Dracocephalum kotschyi* were used for induction of hairy root cultures using the *Agrobacterium rhizogenes* strain A4, by immersion and injection inoculation methods. Furthermore, the effect of different elicitors (colchicines, chitosan and UV-B) on hairy root growth and secondary metabolites were evaluated. Rosmarinic acid production as one of the main active substances of these plants was quantified by HPLC. We were able to induce hairy roots in both plants by immersion method with high transformation frequency (30-50%). Copious growth of hairy roots and secondary metabolites (total flavonoid, total phenolic and antioxidant) production was observed in all of applied elicitors except salicylic acid. Rosmarinic acid production in transformed roots was higher than the amount in non-transformed roots. In the near future, hairy roots will become powerful tools for biotechnologists with which to reach the precious underground resources of the plant kingdom. This review creates a paradigm for future scale up of hairy roots in bioreactors for large scale production of secondary metabolites and its increasing use as model system in metabolic engineering.

Keywords: *Agastache foeniculum*, *Dracocephalum kotschyi*, Elicitor, Hairy root, Secondary metabolaits

Introduction

Agastache foeniculum and *Dracocephalum kotschyi* are important sources of products that people use, including food, cosmetics – sanitary and medicines. Anise hyssop (*Agastache foeniculum*) is a perennial herbaceous plant, belonging to the Lamiaceae family. The origin of this plant has been reported in South America. The essential oil of *A. foeniculum* is used in food, pharmacy, cosmetics – sanitary and ice cream industries. Anise hyssop is also used in traditional medicine for treating lung diseases and cough (Omidbaigi 2007). *A. foeniculum* has many useful constituents including monoterpenes and phenyl propanoids. Rosmarinic acid, chlorogenic acid, rutin, apigenin and galangin, are among the constituents of anise hyssop essential oil, with many pharmacological properties. The antioxidant activity of essential oils depends on the content of total polyphenols, which is measured 98.6 mg/g in *Agastache*

foeniculum (Matei 2012). *Dracocephalum kotschyi* Boiss (Labiateae) is an herbaceous, perennial and endemic plant known in Iran as Zarrin-Giah. Recent pharmacological studies have confirmed some of the methoxylated flavonoids in plant's parts having anti-cancer properties. *Agrobacterium rhizogenes* mediated hairy root production is a valuable tool for studies on the biosynthesis of secondary metabolites and for exploitation in metabolic engineering. For this reason, hairy roots have been induced in several medicinal and aromatic plants and cultured for the production of secondary compounds (Bonhomme et al, 2000). Several factors affect the rate of *A. rhizogenes* mediated transformation (Cao et al, 2009). Many studies have shown that various plant tissues have different responses to transformation with different strains of *A. rhizogenes* (Sharafi et al, 2013; Samadi et al, 2013). The use of biotic and abiotic elicitors is a common strategy that is used to increase the growth rate and production of secondary metabolite in hairy root systems (Soleimani et al, 2012). Rosmarinic acid (RA) is a phenolic compound and is produced in the roots of *Agastache foeniculum* and *Dracocephalum kotschyi* that has shown antioxidant, anti-virus, antimicrobial, anti-allergic, anti- inflammatory and anticancer activities. In the present study two medicinal plants namely *Agastache foeniculum* and *Dracocephalum kotschyi* were chosen to induce the hairy root cultures, as these have medicinal properties residing in the roots. The objective of the study was to find the induction efficiency of hairy roots by *Agrobacterium rhizogenes* in all the two plants. The growth dynamics and secondary metabolites of cultured transformed root were investigated.

Materials and Methods

Explants Preparation

Seeds were obtained from the plants grown in research field of horticultural department, Urmia University, Iran. Seeds were surface sterilized and sterile seeds were cultured on MS (Murashige & Skoog 1962) media containing 7 g L−1 agar. Hypocotyls, cotyledons, internode, one-month old and five-month-old leaves were prepared as explants for transformation.

Bacterial Culture Preparation and plant transformation

Agrobacterium rhizogenes strain A4 (provided by the bank of microbes at the National Institute of Genetic Engineering and Biotechnology (NIGEB),Tehran, Iran) was cultured on LB solid medium for activation of the strain and sub cultured three times on the same medium.The explants were infected with bacteria using two different methods, a) by immersion the leaves in a suspension of bacteria, and b) by injection of bacteria in different parts of explants by 0.5 ml insulin syringes (Exel – America). After infection explants were cultured in MS solid medium supplemented with 3% sucrose in dark regime at 25ºC for 48 hrs. Elimination of bacteria was then done by transferring the stabbed explants to MS solid medium with antibiotic cefotaxime (500 mg/l) at 25ºC in dark. Hairy Roots that developed were transferred to antibiotic and hormone free ½ MS liquid medium at 25ºC at 100 rpm on orbital shaker under dark and sub-cultured in fresh medium every 15 days.

Treatment of hairy roots in liquid media

In separate experiment, the effects of different concentrations of Chitosan (50, 100 and 150 mg L−1), colchicines (0/01, 0/03 and 0/05 %) and UV-B radiation (3, 6 and 9 min) at a week in the hairy roots formation and elicitation of total flavonoid, total phenolic and antioxidant production were investigated. Approximately 1 g of hairy roots were incubated in 250 mL Erlenmeyer flasks containing 50 mL liquid ½ MS medium for 3 months on the rotary shaker (100 rpm) at 25◦C. Liquid ½ MS medium was used in all treatments as control sample.

Preparation of extracts

Lyophilized and powdered plant materials (0/1 g) were separately extracted with 80 % methanol (5 mL). The extraction procedure was the same as that described above. Total

phenolics were determined colorimetrically by the Folin-Ciocalteu method. The prepared extracts (1 ml) were mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted tenfold with distilled water) and allowed to stand at room temperature for 10 min. A 4 ml sodium bicarbonate solution (75 $g.l^{-1}$) was added to the mixture. After 30 min at room temperature, absorbance was measured at 765 nm using a UV spectrophotometer (Pharmacia Biotech).

Determination of total flavonoid content

Total flavonoid content was determined using aluminium chloride (AlCl3) according to a known method, 15 using quercetin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 5% NaNO2 (0.03 ml). After 5 min at 25°C, AlCl3 (0.03 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 380 nm.

Estimation of total antioxidant capacity

The antioxidant activity of extracts were measured by the DPPH (2, 2′-diphenyl-1-picrylhydrazyl) free radical scavenging method. Sample solutions (50µl) in methanol were added to DPPH methanol solution (2 ml, 40 $\mu g.ml^{-1}$). The mixtures were incubated at room temperature for 30 min and the absorbance was measured at 517 nm.

Determining the amount of Rosmarinic acid

High performance liquid chromatography (HPLC) was used for determination of Rosmarinic acid content. Rosmarinic acid was detected at 254 nm. Retention time of Rosmarinic acid was 5.34 min. Chromatographic peaks were confirmed by retention time and UV spectra that are related to the reference standard. The retention time of the peak obtained with standard of Rosmarinic acid was used to identify the corresponding peaks of root extracts.

Results and discussion

Effect of Explants types

Hairy roots were induced at the wound site of explants after 3–4 weeks of culture. There were no hairy roots formed from control explants. In *Agastache foeniculum*, the highest rate of hairy root induction (51.1 %) was obtained for one-month-old leaf explants by A4 strain. The hypocotyl explants showed necrosis with a low rate of hairy root induction. Moreover, hairy roots were not observed in control explants. In *Dracocephalum kotschyi* the one-week-old leaf explants (36/66 %) were highly and significantly susceptible to infection by A4 strain of *A. rhizogenes* and internode explants (1/66 %) showed low rate of hairy root induction. Many studies have shown that *A. rhizogenes* strains and explants types have significant impacts on the induction of hairy roots (Sharafi et al.2013; Soleimani et al. 2012).

Effect of inoculation method on hairy roots induction

Effects of the inoculated methods in hairy roots induction in two plants were different. In *A. foeniculum* ANOVA results indicated no statistically significant differences between the two inoculated methods, but immersion was the best method to inoculation of *D.kotschyi*. Contact between bacteria and plant cells can be increased by direct injection of bacterial suspensions into the explants or by immersion (Tomilov et al., 2007).

Effect of elicitation on hairy root growth and total flavonoid, total phenolic and antioxidant

Investigation the effects of UV-B on the growth rate of hairy roots showed that Fresh weight of hairy roots (1/3 g) declined sharply in 9 min and UV- B treatments were no significant effect on dry weight of roots (fig. 1). Also the highest increase in metabolites was observed at 6 min (fig. 2, 3, 4)

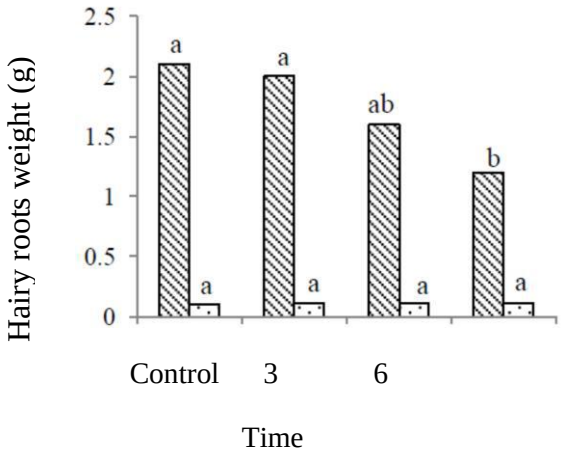

Fig. 1: Effects of UV-B treatments on hairy roots growth. The different letters denote a statistically significant difference at $P \leq 0.05$, as determined by Duncan's multiple

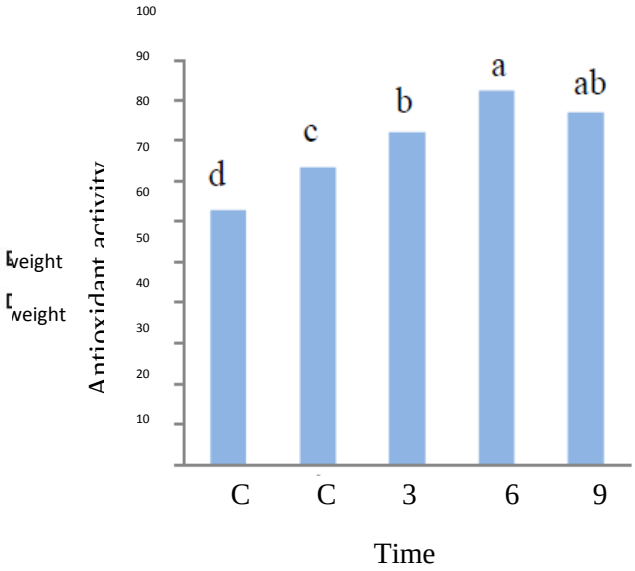

Fig 2: Effects of UV-B treatments on antioxidant activity. The different letters denote a statistically significant difference at $P \leq 0.05$, as determined by Duncan's multiple range test.

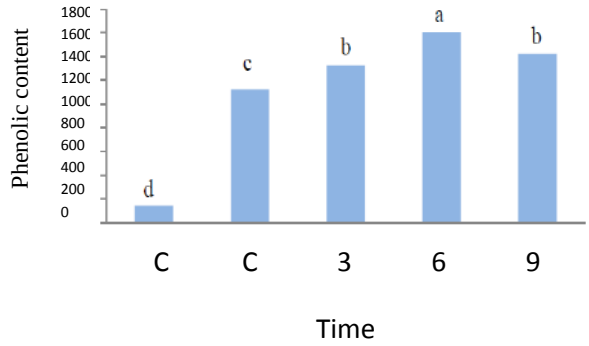

Fig 3: Effects of UV-B treatments on phenolic content. The different letters denote a statistically significant difference at P ≤ 0.05, as determined by Duncan's multiple range test.

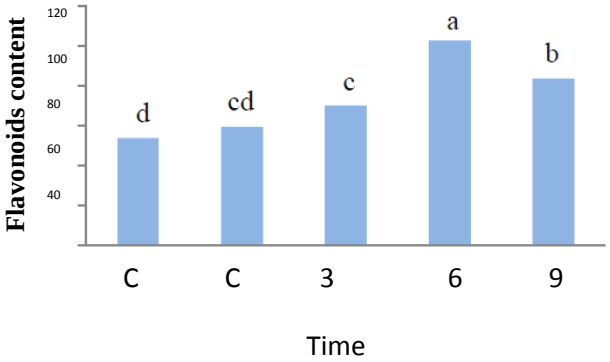

Fig 4: Effects of UV-B treatments on flavonoids content. The different letters denote a statistically significant difference at P ≤ 0.05, as determined by Duncan's multiple range test.

Amongst the various concentrations of colchicine, the 0/05% concentration increased the antioxidant activity (90/33 %), phenolic content (1632/61 µg/g DW) and flavonoid content (143/50 □g/g DW) of the hairy roots (Fig. 5, 6, 7).

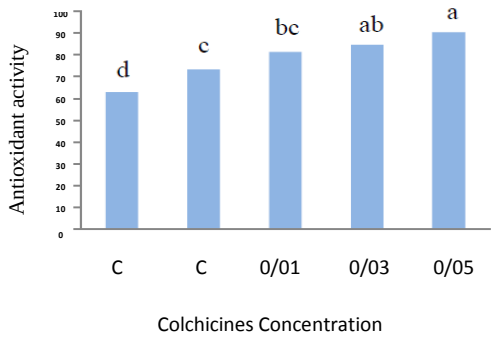

Fig 5: Effects of colchicines treatments on antioxidant activity. The different letters denote a statistically significant difference at P ≤ 0.05, as determined by Duncan's multiple range test.

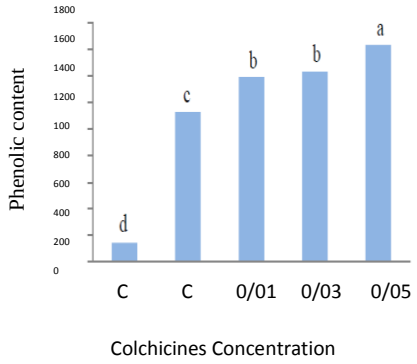

Fig 6: Effects of colchicine treatments on phenolic content. The different letters denote a statistically significant difference at P ≤ 0.05, as

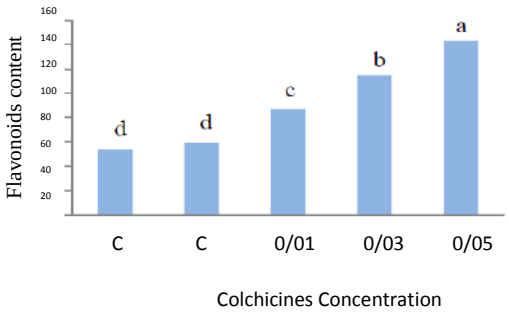

Fig 7: Effects of colchicine treatments on flavonoids content. The different letters denote a statistically significant difference at P ≤ 0.05, as determined by Duncan's multiple test.

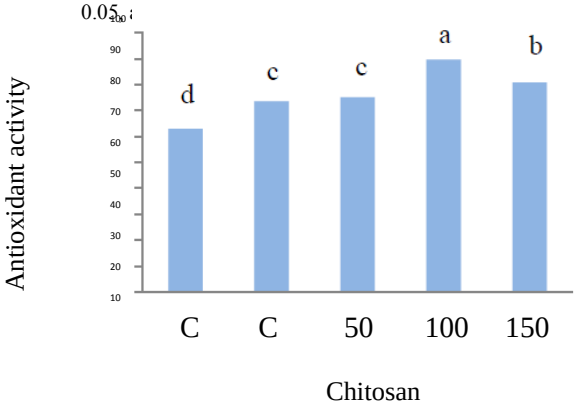

Fig 8: Effects of chitosan treatments on antioxidant activity. The different letters denote a statistically significant difference at P ≤ 0.05, as determined by Duncan's multiple range test.

Chitosan 100 mg/l increased the fresh weight of the hairy roots (fig. 12). The 100 mg/l concentration increased the antioxidant activity (89/66 %), phenolic content (1617/85 µg/g

DW) and flavonoid content (96/33 µg/g DW) of the hairy roots (Fig. 8, 9, 10). Effect of elicitation depends on the elicitor type, environmental conditions, the concentration of elicitor and the interaction between the elicitor and plant cells (Smetanska 2008).

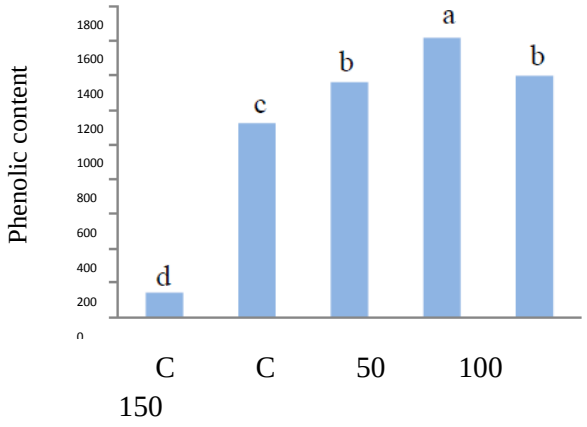

Fig 9: Effects of chitosan treatments on phenolic content. The different letters denote a statistically significant difference at $P \leq 0.05$, as determined by Duncan's multiple range test.

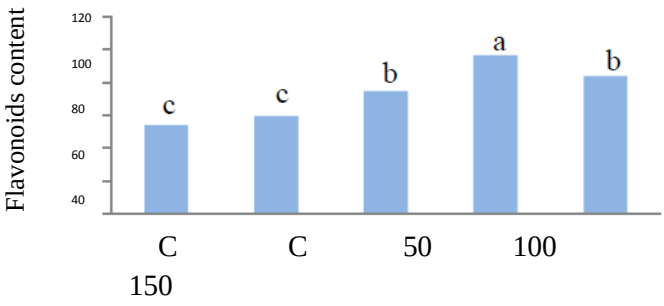

Fig 10: Effects of chitosan treatments on flavonoids content. The different letters denote a statistically significant difference at $P \leq 0.05$, as determined by Duncan's multiple test.

Rosmarinic acid production in hairy roots

The HPLC analysis confirmed the presence of RA in tested samples according to the same retention time of 5.34 min as standard (fig. 11). The highest amount of RA (213.42 µg g−1 dry weight) was produced in transformed roots; the level was almost 4-fold higher than RA content of non-transformed roots (52.28 µg g−1 dry weight) under the same conditions. Many studies have pointed out that hairy root growth and metabolite production depend on '*rol*' gene expression, the *rol* genes could activate expression of defense genes and thus the production of secondary metabolites (phytoalexin production) in plant cells (Sherafi et al. 2013).

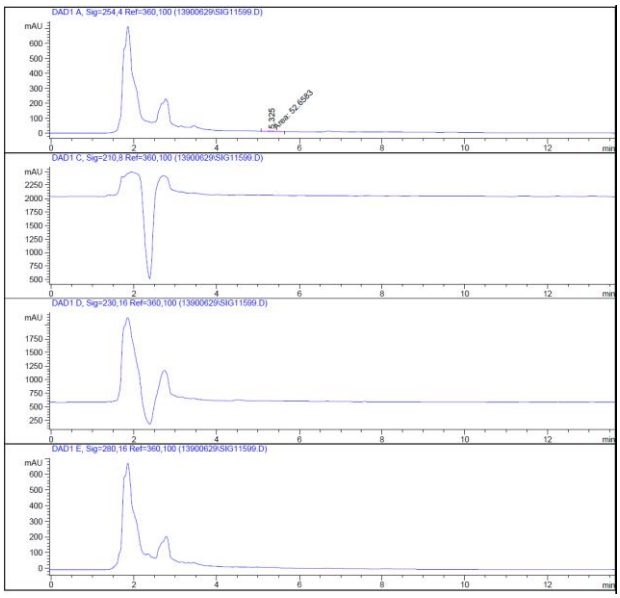

Fig. 11: Rosmarinic acid content of hairy root cultures, grown in 1/2 MS media. Values represent the mean ± SD of three independent measurements.

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PLANT PROTECTION AND FOOD SAFETY

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STUDY OF OCHRATOXIN A' AND ZEARALENONE' CONTAMINATION IN WHEAT AND MAIZE FROM ALBANIA

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Abstract

Ochratoxin A (OTA) and Zearalenone (ZON) are mycotoxins, natural contaminants, produced by molds. OTA is produced by *Aspergillus ochraceus* and *Penicillium verrucosum* species, while ZON is produced by *Fusarium* species. Exposure to OTA has been associated with distinct renal diseases in humans endemic in the Balkans, referred to Balkan Endemic Nephropathy (BEN) and Urinary Tract Tumors (UTT). The critical effects of ZON result from its potent oestrogenic activity, considered as a possible causative agent in the outbreaks of precocious pubertal changes in young children. The objective of this study was to assess the presence of OTA and ZON in cereal commodities harvested during 2014 in different regions of Albania and to assess risk of mycotoxin exposure posed to humans and animals. Random flour samples (n = 21): winter wheat (14 samples) and maize (7 samples) were collected in Lushnja - Fieri region (n = 14) and Korça region (n = 7), according to their production distribution in the country. The samples were analyzed, by High Pressure Liquid Chromatography (HPLC), coupled with fluorescence detector. Analytical results indicate that OTA levels in two maize samples (139.15 and 8.575 μg/kg), was above the Maximum Residue Level (MRL) established by Commission Regulation 165/2010 amending EU Regulation 1881/2006, for maize (5 μg/kg) with destination for human consumption, while ZON levels detected was under MRL (200 μg/kg), established on wheat for human consumption. Our findings indicate that OTA and ZON presence in cereals harvested in Albania is established, and may indicate health implications to consumers.

Key words: *Ochratoxin A*, *Zearalenone*, *Wheat*, *Maize*, *Albania*.

Introduction

Mycotoxins are secondary metabolites of fungi. Due to the widespread distribution of fungi in the environment, mycotoxins are considered to be one of the most important contaminants in foods and feeds. According to the Food and Agriculture Organization (FAO), more than 25% of the world's agricultural production is contaminated with mycotoxins, resulting in economic losses in the grain industry (Cazzaniga *et al*., 2001). *Aspergillus*, *Fusarium*, and *Penicillium*, all known field fungi, are the natural fungal flora associated with foods. The most common mycotoxins are aflatoxins, deoxynivalenol (DON), zearalenone (ZON), ochratoxin A (OTA), fumonisin, and patulin (Gaag *et al*., 2003).

Ochratoxins are a group of mycotoxins produced by several fungi of the *Aspergillus* or *Penicillium* families and are weak organic acids consisting of a derivative of an isocoumarin. The family of Ochratoxins consists of three members, A, B, and C, which differ slightly from each other in chemical structures. These differences, however, have marked effects on their respective toxic potentials. Ochratoxin A is the most abundant and hence the most commonly detected member but is also the most toxic of the three members (Van der Merwe et al., 1965). OTA is produced by *Aspergillus ochraceus* and *Penicillium verrucosum* species. Exposure to OTA has been associated with distinct renal diseases in humans endemic in the Balkans (in Bulgaria and ex-Yugoslavia), referred to as Balkan Endemic Nephropathy (BEN) and Urinary Tract Tumors (UTT) (Stoev et al., 1989). Ochratoxin A (OTA) is a nephrotoxin,

affecting all tested animal species, though effects in man have been difficult to establish unequivocally. It is listed as a probable human carcinogen (Class 2B) (Olsen et al., 2006).
Zearalenone was discovered as the cause of a reproductive disorder in pigs known as vulvovaginitis (Moss, 2002). ZON is a oestrogenic mycotoxin that can be produced by several field fungi including *Fusarium graminearum* (*Gibberella zeae*), F. *culmorum*, F. *cerealis*, F. *equiseti* and F. *semitectum* (Bennett and Klich, 2003). ZON is one of the most common *Fusarium* mycotoxins in the temperate regions of America, Europe and Asia. It is most frequently encountered on corn, but also contaminates other cereals and plant products (Krska *et al*., 2003). The critical effects of ZON result from its potent oestrogenic activity, considered as a possible causative agent in the outbreaks of precocious pubertal changes in the young children. The International Agency for Research on Cancer (IARC) has categorized ZON as a class 2A carcinogen (IARC, 1993).
In this study we have analyzed the ochratoxin A and zearalenone contamination in cereals harvested during 2014 in different regions of Albania. Random samples (n = 21): winter wheat (14 samples) and maize (7 samples) were collected according to EU regulation 401/2006. The wheat and maize samples collected directly after their harvesting season avoided the post-harvest contamination stage, which indicate that OTA and ZON origin of contamination in cereals is of pre-harvest stage.
The wheat and maize samples were collected in Lushnja - Fieri region (n = 14) and Korça region (n = 7), according to their production distribution in the country. The objective of this study was to assess the risk of mycotoxin exposure posed to humans and animals. The samples were analyzed, separately for OTA and ZON contamination by High Pressure Liquid Chromatography (HPLC) (LOD = 0.2 μg/kg).

Materials and methods

Sample characterization

Flour samples of winter wheat and maize were collected during 2014 in mill factories from three different regions of Albania: Korça (wheat and maize), Lushnja and Fieri (only wheat). Selection of these regions was based on the statistics for agriculture production in country (INSTAT, 2014). As the distribution of mycotoxins is not uniform throughout the matrix, the sampling procedure was carried out according to Commission Regulation (EC) No 401/2006 (European Commission, 2006).

Mycotoxins analysis

Apparatus

Linear shaker IKA HS 501 digital (IKA Labortechnik, Germany) was used for the extraction. For liquid chromatography, the system Waters Alliance 2690 with the fluorescence detector Waters 474 (Waters, MA, USA) equipped with columns Prodigy 5μ ODS(2) was used for OTA and ZON detection.

Chemicals and reagents

Standard solutions of OTA and ZON were purchased from Biopure (Tulln, Austria). For sample clean-up, immunoaffinity columns (R-Biopharm Rhône) were used. Reagents purchased at Merck (Darmstadt, Germany) and Supelco (PA, USA) were of analytical or chromatography grade purity.

Analytical procedure

OTA and ZON mycotoxins were determined separately in a single HPLC method following the instruction for use enclosed to respective immunoaffinity columns and standards (R-Biopharm Rhône, 2003). Mycotoxins were extracted from samples with appropriate solvents.

After sample clean-up, mycotoxins were determined by liquid chromatography. The limit of detection (LOD) and the limit of quantification (LOQ) for OTA and ZON was 0.2µg/kg. The results of were calculated according to sample moisture content of 12%.

Results and discussion

Two cereal grains, wheat and maize, are the most important cereal crop in terms of cultivated area, production, and consumption in the world as well in Albania. Owing to the abundant production and the main role of wheat and its flour products in the diet of humans and animals, they can play a very important role in endangering human health in case of contamination with health-threatening factors. In the farm and the warehouse, wheat can be invaded by different microorganisms, especially fungi (Saari *et al*., 1968).

Table 3. Levels of Ochratoxin A and Zearalenone in wheat samples in Lushnja and Korçë regions of Albania

Code	Regions	Ochratoxin A (µg/kg)	Zearalenon (µg/kg)
1	Lushnjë	<LOD	<LOD
2	Lushnjë	<LOD	<LOD
3	Lushnjë	<LOD	<LOD
4	Lushnjë	<LOD	<LOD
5	Lushnjë	<LOD	<LOD
6	Lushnjë	<LOD	<LOD
7	Lushnjë	<LOD	<LOD
8	Korçë	2.816	<LOD
9	Korçë	<LOD	<LOD
10	Korçë	<LOD	<LOD
11	Korçë	<LOD	<LOD
12	Korçë	<LOD	<LOD
13	Lushnjë	<LOD	<LOD
14	Lushnjë	<LOD	<LOD
Mean value/positive samples		**2.816**	**-**
Min Value		**2.816**	**-**
Max Value		**2.816**	**-**
Incidence of positive samples		**7.1 %** (1/14)	**0.0 %** (0/14)
Above MRL (%)		**0.0 %** (0/14)	**0.0 %** (0/14)

Table 2. Levels of Ochratoxin A and Zearalenon in maize samples in Lushnja, Fieri and Korçë regions of Albania

Code	Regions	Ochratoxin A (µg/kg)	Zearalenone (µg/kg)
1	Korçë	<LOD	<LOD
2	Fier	139.15	<LOD
3	Fier	<LOD	<LOD
4	Fier	8.575	<LOD
5	Korçë	<LOD	<LOD
6	Lushnjë	<LOD	<LOD
7	Lushnjë	<LOD	37.806
Mean value/positive samples		**73.8625**	**37.806**
Min Value		**8.575**	**37.806**
Max Value		**139.15**	**37.806**
Incidence of positive samples		**28.6 %** (2/7)	**14.3 %** (1/7)
Above MRL (%)		**14.3 %** (1/7)	**0.0 %** (0/7)

OTA was detected in two out of six maize samples varying 8.575-139.15 µg/kg, while in wheat was only one out of 14 samples in concentration 2.816 µg/kg. ZON was detected only in one maize sample (37.806 µg/kg). Analytical results indicate that OTA levels in two maize samples was above the Maximum Residue Level (MRL) established by Commission Regulation 165/2010 amending EU Regulation 1881/2006, for maize (5 µg/kg) with destination for human consumption, while ZON levels detected was under MRL (200 µg/kg), established on wheat for human consumption. Our findings indicate the OTA and ZON presence in cereals harvested in Albania is established, and may indicate health implications to consumers.

Conclusion

After analysis performed by HPLC, some of the wheat and maize samples resulted being contaminated with Ochratoxin A and Zearalenone. It is noticed that the incidence of positive samples in maize samples were bigger than in wheat samples. It is worth noting that in maize samples, we found samples containing higher OTA values than maximum limits as defined by national and European legislations. From this experimental work, we conclude that the risk of the preharvest contamination of OTA and ZON in cereals, in Albania, is present, especially for the maize produced during 2014.

Considering all the problems that cause contaminated cereals with mycotoxins (OTA and ZON) in human and animal health, it is recommended to make systematic analyses, because cereals are the basic food of population in Albania. Being the basic food of the population, increases the possibility of exceeding the daily limits that everybody (human and animals) should consume.

Acknowledgements

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THE IDENTIFICATION OF MOLD DEVELOPED IN WHEAT'S GERM OBTAINED FROM INDUSTRIAL MILL COMPANIES IN KORÇA, ALBANIA

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Abstract

Cereals and cereal products are significant and important human food resources and livestock feeds worldwide. Cereal grains are food staples in many countries. They are the raw materials of many our foods and certain beverages. Because of their extensive use as human foods and livestock feeds, the microbiology and safety of cereal grains and cereal products is a very important area. The aim of this study is the identification of mold developed in wheat's germ. The samples were obtained in sterile condition from 5 different industrial mill companies in south-east of Albania during March- June period 2014. The wheat was treated with 90ml neutral detergent 0.1 %. Then it was washed four times with 90ml of sterile water. The kernels were dried in Petri dishes with filter paper which were sterilized before. The identification of mold was determined by the method of placing the wheat's germ on solid medium Czapek and Potato dox agar. It was cultivated a total of 10 plates with 5 kernels for each medium. The Petri dishes was incubated in 25^0C. The identification of mold was based on colony growth, cultural and phenotypic characteristics. Mold's species present in wheat were determined by direct microscopic observation. By identification of moulds and classification in species and genus there was noticed mainly Ascomycete classes especially Aspergillus and Penicillium species. Ascomycetes class mold are characteristic of stored grains. This shows that wheat used in industrial mill companies are not young, but are stored wheat for a while. In general wheat samples which have been analyzed were within standards.
Keywords: *microbiological characteristics, wheat, mold, Aspergillus, Penicillium, species.*

Introduction

Wheat is probably the most common cereal available all over the world and is in even higher demand in recent years due to its abundant health benefits. Over the years, wheat has shown itself to be one of the most successful and sustainable cereals crops in the world.

Because of their extensive use as human foods and livestock feeds, the microbiology and safety of cereal grains and cereal products is a very important area. The sources of microbial contamination of cereals are many, but all are traceable to the environment in which grains are grown, handled, and processed.

Microorganisms that contaminate cereal grains may come from air, dust, soil, water, insects, rodents, birds, animals, humans, storage and shipping containers, and handling and processing equipment. Many factors that are a part of the environment influence microbial contamination of cereals, including rainfall, drought, humidity, temperature, sunlight, frost, soil conditions, wind, insect, bird and rodent activity, harvesting equipment, use of chemicals in production versus organic production, storage and handling, and moisture control. The microflora of cereals and cereal products is varied and includes mold, yeast and bacteria.

This study was based on fungus present in wheat's germ. Total numbers of fungi was determined by dilution techniques (Pitt, J. et al, 2009). The samples were obtained in strictly sterile conditions in order to avoid the impact of environmental and air microorganisms. They were taken in different parts of silos for a better presentation. This was done because microorganisms are different in the upper and inner layer of silos (King, A.D. et al, 2003).

The total numbers of molds were determined by the method of placing the suspensions on solid medium (Czapek dox agar, Potato dox agar, Malt extract agar, Plate count agar). It was prepared three dilutions with 3 parallels. After incubation at 26°C the colonies were counted. The calculation of total number of microorganisms was done by assuming that each colony grew from a single cell (Prifti, D. et al, 1980).
A very important point of this study was the identification of molds that were grown in all media. The identification of mold was based on colony growth, cultural and phenotypic characteristics. Some of them were determined by direct microscopic observation. They were classified in 5 species: *Rhizopus spp.* and *Mucor spp*. (Pitt, J. et al 2009), *Aspergillus spp*. (Adolf, J. et al, 1970), *Penicillium spp*. (Raper, B.K. et al, 1992), *Fungi imperfecti spp*. (Barnet, H.L. et al, 1972).

Materials and methods

The aim of this study is the identification of mold developed in wheat's germ. The samples were obtained in sterile condition from 5 different industrial mill companies in south-east of Albania during March-June period 2014. Experimental work has been developed in the laboratory of microbiology at the Faculty of Natural Sciences of Tirana, Albania.
Experimental work was based on :
1kg of wheat was taken for each sample in order to prepare the sample average with diagonal division. It was analyzed 5 samples. The wheat kernels were treated with 90ml neutral detergent 0.1 %. They were washed four times with 90ml of sterile water. Then they were shaken for 1 minute. The kernels were dried in Petri dishes with filter paper which were sterilized before. The identification of mold was determined by the method of placing the wheat's germ on solid medium Potato dox agar and Czapek dox agar This was done by using a forcep. It was cultivated a total of 10 plates with 5 kernels for each medium. The Petri dishes was incubated in 25^0C. The identification of mold was based on colony growth, cultural and phenotypic characteristics. Mold's species present in wheat were determined by direct microscopic observation.

Results and discussion

As mentioned in the methodology, 5 samples were analyzes and total of 10 plates were cultivated with 5 kernels for each medium. It was determined the number of kernels with mold, their percentage and the total number of mold developed in each Petri dishes.
The results are presented in Table 1 for cultivated kernels in Potato dox agar and Czapek dox agar medium. These results are presented graphically for a clearer result (Figure 1). Sample which represented the highest percentage of kernels with mold was sample 1 for both media. This rezult is too high for this sample (72% in PDA dox agar and 82% in Czapek dox agar) and this shows that the wheat's germ is a sutable plase for mold's development.

Table 1 : Cultivated kernels in Potato dox agar and Czapek dox agar medium

Samples	Nr. of kernels with mold		% of kernels with mold		Total nr. of Mold	
	PDA	Czapek	PDA	Czapek	PDA	Czapek
Sample 1	36	41	72%	82%	41	50
Sample 2	18	24	36%	48%	24	30
Sample 3	29	35	58%	70%	31	38
Sample 4	34	18	68%	36%	36	20
Sample 5	31	34	62%	68%	40	46

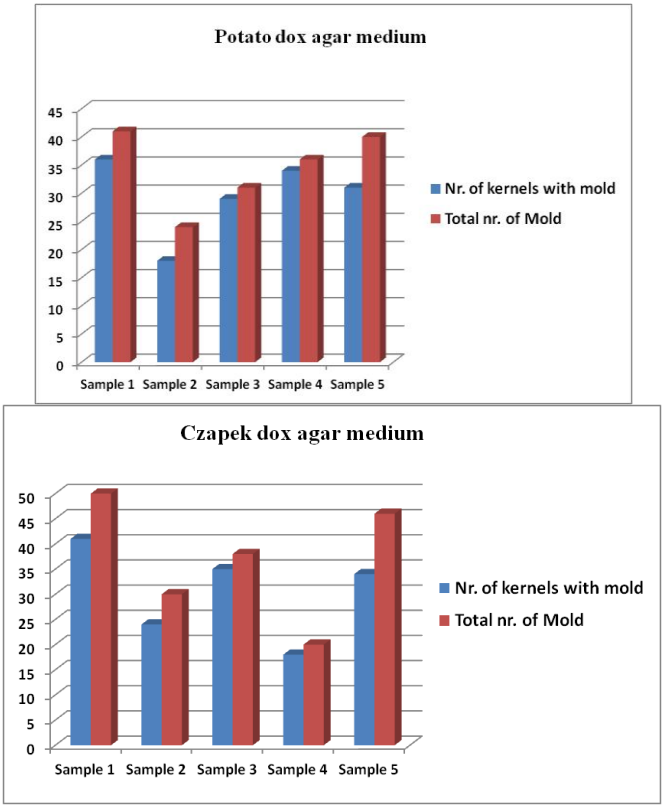

Figure 1 : Number of kernels with mold and Total number of mold cultivated in Potato dox agar and Czapek dox agar.

A very important point of this study was identifying the molds that were grown in all media. The identification of mold was based on colony growth, cultural and phenotypic characteristics. Some of them were determined by direct microscopic observation.

By identification of mold and classification in species and genius there was noticed mainly Ascomycete class especially Aspergillus and Penicillium species. Ascomycetes class mold are characteristic of stored grains. This shows that wheat used in industrial mill companies are not young, but are stored wheat for a while.

In general wheat samples which have been analyzed were within standards. The results are presented in, Figure 2, Table 2 and 3. The sample with the highest number of mold, especially *Aspergillus spp*. was sample 1.

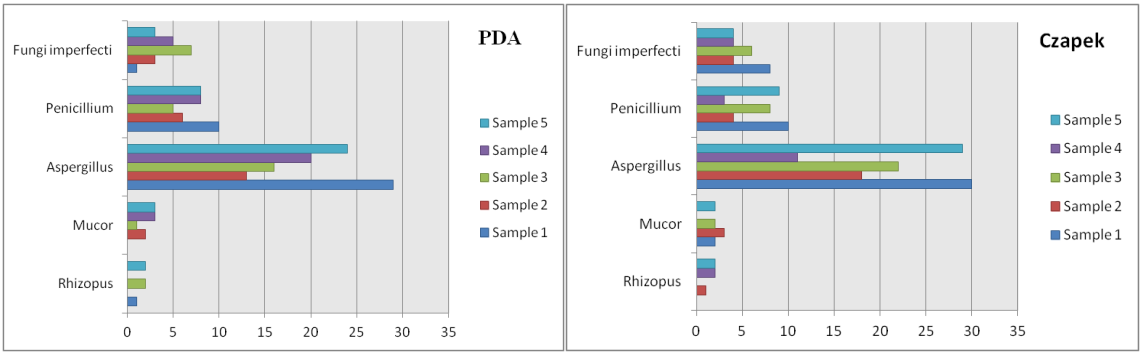

Figure 2 : Distribution of mold according to Potato dox agar and Czapek dox agar

Table 2 : The identification of mold in wheat's germ cultivated in Potato dox agar

Class	**Phycomycetes**		**Ascomycetes**		**Fungi imperfecti**
Genius	Rhizopus	Mucor	Aspergillus	Penicillium	
Samples					
Sample 1	*R.nigricans* 1		*A.candidus* 11	*Penicillium spp.* 5	*Cladosporium spp.* 1
			A.flavus 14	*P.rubrum* 1	
			A.terreus 1	*P.islandicum* 4	
			A.versicolor 1		
			A.niger 2		
Total	**1**	**0**	**29**	**10**	**1**
Sample 2		*Mucor* 2	*A.candidus* 4	*Penicillium spp.* 6	*Trichoderma spp.* 1
			A.flavus 1		*Helminthosporium spp.* 2
			A.terreus 3		
			A.niger 1		
			A.versicolor 4		
Total	**0**	**2**	**13**	**6**	**3**
Sample 3	*R.nigricans* 2	*Mucor* 1	*A.candidus* 6	*Penicillium spp.* 3	*Helminthosporiumspp.* 3
			A.flavus 2	*P.rubrum* 2	*Cladosporium spp.* 3
			A.niger 2		*Trichoderma spp.* 1
			A.versicolor 2		
			A.terreus 4		
Total	**2**	**1**	**16**	**5**	**7**
Sample 4		*Mucor* 3	*A.candidus* 8	*Penicillium spp.* 2	*Helminthosporium spp.* 2
			A.flavus 4	*P.rubrum* 4	*Cladosporium spp.* 2
			A.terreus 4	*P.islandicum* 2	*Trichoderma spp.* 1
			A.versicolor 2		
			A.niger 1		
			A.glaucus 1		
Total	**0**	**3**	**20**	**8**	**5**
Sample 5	*R.nigricans* 2	*Mucor* 3	*A.candidus* 8	*Penicillium spp.* 5	*Cladosporium spp.* 1
			A.flavus 7	*P.rubrum* 1	*Trichoderma spp.* 2
			A.versicolor 3	*P.islandicum* 2	
			A.terreus 3		
			A.niger 3		
Total	**2**	**3**	**24**	**8**	**3**

Table 3 : The identification of mold in wheat's germ cultivated in Czapek dox agar

Class	**Phycomycetes**				**Ascomycetes**				**Fungi imperfecti**	
Genius	Rhizopus		Mucor		Aspergillus		Penicillium			
Samples										
Sample 1			*Mucor*	2	*A.candidus*	18	*Penicillium spp.*	8	*Helminthosporium spp.*	3
					A.flavus	9	*P.rubrum*	2	*Cladosporium spp.*	4
					A.niger	1			*Trichoderma spp.*	1
					A.terreus	1				
					A.versicolor	1				
Total		**0**		**2**		**30**		**10**		**8**
Sample 2	*R.nigricans*	1	*Mucor*	3	*A.candidus*	8	*Penicillium spp.*	3	*Trichoderma spp.*	1
					A.flavus	4	*P.islandicum*	1	*Cladosporium spp.*	1
					A.terreus	4			*Helminthosporium spp.*	2
					A.niger	1				
					A.versicolor	1				
Total		**1**		**3**		**18**		**4**		**4**
Sample 3			*Mucor*	2	*A.candidus*	10	*Penicillium spp.*	2	*Helminthosporium spp.*	4
					A.flavus	8	*P.rubrum*	4	*Cladosporium spp.*	1
					A.versicolor	2	*P.islandicum*	2	*Trichoderma spp.*	1
					A.terreus	1				
					A.glaucus	1				
Total		**0**		**2**		**22**		**8**		**6**
Sample 4	*R.nigricans*	2			*A.candidus*	3	*Penicillium spp.*	3	*Trichoderma spp.*	4
					A.flavus	4				
					A.terreus	1				
					A.niger	3				
Total		**2**		**0**		**11**		**3**		**4**
Sample 5	*R.nigricans*	2	*Mucor*	2	*A.candidus*	13	*Penicillium spp.*	6	*Helminthosporium spp.*	2
					A.flavus	10	*P.rubrum*	2	*Cladosporium spp.*	2
					A.terreus	3	*P.islandicum*	1		
					A.versicolor	1				
					A.glaucus	2				
Total		**2**		**2**		**29**		**9**		**4**

Conclusion

By identification of mold and classification in species and genius there was noticed mainly Ascomycete class especially Aspergillus and Penicillium species. Ascomycetes class mold are characteristic of stored grains. This shows that wheat used in industrial mill companies are not young, but are stored wheat for a while. In general wheat samples which have been analyzed were within standards. From all the experimental work, we may recommend that the industrial mill companies should pay attention to preparation for milling processes, such as : cleaning, sorting, conditioning of the wheat because wheat is the basic food in our life.

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ANALYSIS OF TRANS-FATTY ACIDS AND THE QUALITY OF OLIVE OIL EXTRACTED IN ALBANIA

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Abstract

Olive oil, one of the oldest manmade agricultural products, has retained its status of importance in modern area. The authenticity of the Olive Oil is of great importance both on economic and health aspect. Evaluation of quality is focused in the group of quality indicators such as: Acidity, Absorbance (K_{270}); Stability index, Peroxide value (PV), etc. Unsaturated fatty acids, naturally, are present in *cis* isomers, while under thermal stress during technological processes such as: extraction, refining and bleaching the trans- isomers are formed. Exceeding the minor content levels of 0.05 % per trans-isomers of oleic acid and 0.05% as the sum of the trans-isomers of linoleic and linolenic acid, indicates the authenticity of the product. The *trans*-fatty acids play a key role in development of cardiovascular diseases by increasing the risk of heart diseases and many other diseases.

The Olive oil samples were collected from regions where is assumed main production of Olive oil by Kalinjoti *cv*, and the quality of olive oil were characterized for trans-fatty acids by gas chromatographyapplied for extraction in Olive Mill factories. Fatty acid profiles revealed the presence of 17 FA, including the *trans*-isomers. Trans fatty acids in olive oil samples resulted on values range from 0.01 % to 0.02 %, while in the sum of trans-linoleic acid isomers in maximum value of 0,03 % to 0.05. The sum of *trans*-FA resulted under or equal to 0.05%, indicating the geniunity of the product produced by local Olive Mills.

Keywords*: Extra Virgin Olive Oil, Virgin Olive Oil, fatty acid, trans-isomers, Albania.*

Introduction

Olive Oil (OO), extracted from olive fruits, has been a traditional product of ancient Mediterranean populations. It has been a staple food of the Mediterranean region in the past, while in last decades it has gained popularity, particularly in Western developed countries. The Mediterranean Diet has been connected with low incidence of Cardiovascular Diseases, found in population groups of Cretans (Greece) and Sardinians (Italy), aiming mainly to the use of diet with high levels of polyphenols, originating from Olive oil and Red wine (Patumi *et al*, 2002).

Based on a set of chemical and sensory evaluation analysis, definition and perception of defects, olive oils are classified into three categories; extra virgin olive oil (EVOO), virgin olive oil (VOO) or lampante virgin oil (LVO) (Topi *et al*. 2012). The authenticity of the various Olive oils according to their category is of great importance on economic and health aspect. Evaluation of quality is focused in the group of quality indicators: Acidity (Free Fatty Acids - FFA), Absorption Coefficient (K_{270}); Stability index, Peroxide value (PV), etc. The trans- isomers of oleic, linoleic and linolenic acid are formed by thermal stress as it occurs during refining, bleaching and related processes in different oils. They are a proof for such treatments in case that they exceed the detectable amount of 0.05 % for trans-isomers of oleic acid and 0.05% as the sum of the trans-isomers of linoleic and linolenic acid, indicating for authenticity of Olive Oil (OO). *Trans*-fatty acid play key role in development of cardiovascular diseases by increasing the risk of heart diseases and many other diseases.

Cultivation of olive tree and production of the olive oil in Albania is present mainly in regions of Western part, along the Adriatic and Ionian Sea while is present in the inland river valleys of Shkumbini, Osumi and Vjosa rivers (Shundi *et al*, 2006). Such diversity of regions of Albania, impose production of OO with distinctive characteristics and tastes, mainly due to different olive cultivars, local environment conditions (soil, climate, altitude) and agricultural applied practices.

According to International Olive Oil Council and European Community, the determination of Fatty Acids (FA) is an important quality parameter for Vegetable oils. The International Olive Oil Council (IOOC, 2001) and the EEC (EC, 1991) have defined the quality of olive oil (Table 1), based on parameters that include free fatty acid (FFA) content, peroxide value (PV), Absorption Coefficient (K_{232} and K_{270}), Stability index and sensory score (Bouskou *et al*. 2006; EC, 2003; IOOC, 2003; Vossen *et al*. 2007) .

In particular, the quantity of FFA is an important factor for classifying olive oil into commercial grades. The hydrocarbon chain of fatty acids in OO vary between 12 and 24 carbons (Mailer *et al*. 2006). The hydrocarbon chain is saturated or with one, two or three double bonds :

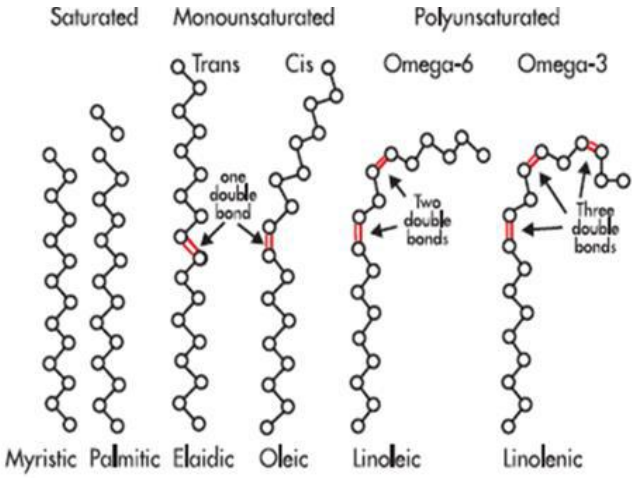

Figure 1: Various types and forms of edible oil fatty acids.

Table 2: Identity characteristics of olive oil categories fixed by IOOC (Boskou *et al*. 2006)

Categoria	Waxex mg/kg	Saturated acids in 2-position of triacyglycerol %	Stigma-stadienes mg/kg	ΔECN42	Trans oleic isomers %	Trans linolei c + trans linolenic isomers %	Cholesterol %	Brassicasterol %	Campesterol %	Sigmasterol %	B-sitoserol %	Δ7-stigmastenol %	Total sterol mg/kg	Erithrodiol+Uvaol %	C14:0 %	C18:3 %	C20:0 %	C20:1 %	C22:0 %	C24:0 %
Extra Virgin olive oil	≤250	≤1.5	≤0.15	≤ 0.2	≤ 0.05	≤ 0.05	≤ 0.5	≤ 0.1	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
Virgin olive oil	≤ 250	≤ 1.5	≤ 0.15	≤ 0.2	≤ 0.05	≤ 0.05	≤ 0.5	≤ 0.1	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
Ordinary virgin olive oil	≤ 250	≤ 1.5	≤ 0.15	≤ 0.2	≤ 0.05	≤ 0.05	≤ 0.5	≤ 0.1	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤0.4	≤0.2	≤0.2
Lampante virgin olive oil	≤300	≤ 1.5	≤ 0.15	≤ 0.3	≤ 0.1	≤ 0.1	≤ 0.5	≤ 0.1	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
Refined olive oil	≤ 350	≤ 1.8	-	≤ 0.3	≤ 0.2	≤ 0.3	≤ 0.5	≤ 0.1	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
Olive oil	≤ 350	≤ 1.8	-	≤ 0.3	≤ 0.2	≤ 0.3	≤ 0.5	≤ 0.1	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
Crude olive residue oil	≤ 350	≤ 2.2	-	≤ 0.6	≤ 0.2	≤ 0.1	≤ 0.5	≤ 0.2	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.3	≤ 0.2
Refined olive residue oil	≤ 350	≤ 2.2	-	≤ 0.5	≤ 0.4	≤ 0.35	≤ 0.5	≤ 0.2	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.3	≤ 0.2
Olive residue oil	≤ 350	≤ 2.2	-	≤ 0.5	≤ 0.4	≤ 0.35	≤ 0.5	≤ 0.2	≤ 4.0	<camp	≥93.0	≤ 0.5	≥1000	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.3	≤ 0.2

In the case that a double bond occurs on the chain, it can orient itself in two ways (Figure 1), the *trans* and *cis* configuration, where the second cause the chain to bend or curve making it to lose the straight structure [Mailer *et al*. 2006]. OO is obtained by different extraction technologies: cold pressing, two-phase, three-phase and recently two and half-phase. According to the extraction technology, the hydrophilic polar compounds may be present in final product in different amounts. Application of lower temperatures during the extraction increase the content rates of, biologically active substances (tocochromanols, phytosterols, carotenoids, phenolic compounds) (Matthaus *et al.* 2003; Schwartz *et al.* 2008; Siger & Nogala-Kałucka 2008; Rusinek et al. 2012).
The cold-pressed oils do not contain any of the isomers of *trans*-FA which are formed in refined oils as a result of the drastic process parameters (Beckingham *et al*. 2006). When oils are exposed to elevated temperatures or pressure they can change from the "cis" (natural isomer) to the "trans" isomer (IOOC, 2003). The separation of the FA especially for *trans*-FA can be achieved on gas-chromatography (GC-FID). Lower grade olive oils and refined olive oil allow wider limits for *trans*-isomers. *Trans* isomers show different physical properties compared to the natural cis isomers and are readily separated with gas chromatography. This method can also detect if oil has been exposed to color removal substances (Wroniak *et al*. 2008).
Nowadays determining the level of *trans*-FA in food is a requirement of the governmental health agencies, since it is necessary to give people information about lipid composition in the food they are eating, especially for those who have health problems like heart disease, obesity and hypertension (Serran *et al*. 2009).
The latest research shows that the main concern is the effect of trans-FA on cardiovascular diseases. *Trans*–FA have been shown to negatively increase the risk for cardiovascular diseases. Consumption of trans-FA: has been shown to increase "bad" LDL cholesterol levels, while decrease "good" HDL cholesterol levels, as well as have adverse effects on the inner lining of blood vessels, and raises the risk of diabetes (Serran et al. 2009).

Materials and Methods

Materials

Twelve samples of mono cultivar olive oils were collected (December 2014) by the same cultivar but from different region (Vlora and Mallakastra region and Kavaja-Vora region), aiming to give indication of the origin of product through studying fatty acid (FA) profiles and trans fatty acids. The samples were characterized for their trans fatty acids by a lot of methods and limits, which were introduced into the International Olive Oil Council (IOOC) trade standards (IOOC, 2001), into EC Regulation 2568/91 (EC, 2003) and into the Codex Alimentarius Standard (Codex Alimentarius, 1981) for controlling product authenticity and quality (Bianchi *et al*,.).
The conditions for the analysis were: solvent: acetone/acetonitrile 50:50 v/v, flow rate 1.2 mL/min., oven temperature 42^0C.

Methods-Determination of Trans-Fatty Acid (TFA)

Fatty acid methyl esters (FAME) were prepared through direct alkaline transesterification, according to the method proposed by IOOC, using pentadecanoic acid (C 15:0) as internal standard.
The mixture FAME prepared was analyzed by a model of gas – chromatography, with flame ionization detector (FID) using a capillary fused silica column containing a cyanopropylsilicone stationary phase (50 m × 0.25 - 0.32 mm × 0.1 - 0.30 μm). The

chromatographic conditions were optimized, including for *trans*-fatty acids analysis, with a programmed column temperature: 150 – 200 °C. The temperature of injector and detector was set at 250 °C and 260 – 280 °C, the carrier gas was hydrogen or helium (1.2 ml/minute) and quantity of substance injected: 1µl of solution at 2% in hexane (IOOC, 2001; Karn *et al*. 2013).
Calculations were performed according to AOCS Official Method Ce 1b-89 (AOCS, 1994), and by the computer program according the EC Regulation 2568/91 method (EC, 2003). According to this method, the trans fatty acid content of olive oil is given by the sum of the following acid contents relative of the total fatty acids (IOOC, 2001):
trans – octadecenoic (T 18:1)
trans, trans – octadecadienoic (TT 18:2)
cis – trans and trans – cis octadecadienoic [(CT + TC) 18:2]
trans – cis – trans , cis – cis – trans, cis – trans – cis and trans – cis – cis octadecatrienoic [(TCT + CCT + CTC + TCC) 18:3].

Results and Discussion

This study indicates that there is a relationship between olive cultivars and their fatty acid profiles. Fatty acid composition of all the analyzed monovariety oils meets the limits set for extra virgin olive oil (EC Regulation, 2003), because fatty acid composition is used only for the evaluation of oil purity, which is guaranteed by establishing the maximum content of some minor fatty acids. Thirteen different fatty acids (FA) were found for both OO. In order of abundance, the most important ones were: palmitic acid (16:1), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3). Results of analysis of oil from cultivar's of Vlora's and Mallakastra's are shown in the table below (Table 2), indicating the variation in the four most prominent fatty acids in olive oil.

Table 4: Fatty acid profile (in% of total FA) of the Kalinjoti Cultivar

Chemical formula	Kalinjoti (Mallakastra)	Kalinjoti (Vlora)	CODEX STAN 33-1981
C16:0	8.82	11.09	7.5 - 20.0
C16:1	0.45	0.72	0.3 - 3.5
C18:0	2.92	2.57	0.5 - 5.0
C18:1	76.15	75.51	55.0 - 83.0
C18:2	9.31	8.15	3.5 - 21.0
C18:3	0.86	0.75	-

Table reports the percentage of fatty acids in monovariety oils from cultivar Kalinjoti in region of Vlora and Mallakastra and confirm their similar. These results show that there is no big difference between the same cultivar although it is cultivated in different regions of Albania. The difference between the two oils is very small, maybe as a result of the climatic, geographic, genetic and other factors. All the values of fatty acids are almost in conformity to those of International Olive Oil Council's regulation (IOOC, 2001) and Codex Alimentarius (1981).
The fatty acid composition is a quality parameter and authenticity indicator of virgin olive oils (Zambiazi et al. 2007). As shown in Table 3, numerous cis–trans isomers of fatty acids were detected in the both oil samples produced from important olive varieties. The total trans-fatty acid in both oils ranged almost in the limits set for extra virgin olive oil (Table 3). The sum of *trans*-FA resulted under 0.05% in the both oils. This study is a comparison of the *trans* – FA content of the same cultivar but in different region of Albania. The *trans* – FA C18:1T content was: 0.016% (Kalinjoti of Mallakastra) and 0.016% (Kalinjoti of Vlora); *trans* – FA

C18:2T content was: 0.012% for Kalinjoti of Mallakastra and 0.013% for Kalinjoti of Vlora; trans – FA C18:3CTC content was: 0.023% for Kalinjoti of Mallakastra and 0.019% for Kalinjot of Vlora.

Table 3: Trans-Fatty acid content in monocultivar Olive oils of the Kalinjoti Cultivar

Trans fatty acid	Kalinjoti (Mallakastra)	Kalinjoti (Vlora)	CODEX STAN 33-1981
C18:1T	0.016	0.016	0.0 - 0.05
C18:2CT	0.012	0.013	-
C18:3CTC	0.023	0.019	-
C 18:2 CT + C 18:3 CTC	0.035	0.032	0.0 - 0.05
∑TSF	0.051	0.048	0.0 - 0.05

Elaidic acid and total trans-isomers of linoleic and linolenic acids in early harvest groups ranged from 0.00 to 0.02%, respectively. The oils contained small amounts (0.05 %) of total trans-FA. The total amount of trans-fatty acids in virgin olive oils should be not exceed 0.1%. The all trans-FA acid isomers C18:1T and C18:2T + C18:3T were generally within acceptable limits of the IOOC's regulation (IOOC, 2001), the EU (EC, 2003).

The *trans*-isomer fatty acid in an olive oil from the both region is similar with a little difference, result a value from 0.01% to 0.02% and in the sum of trans-linoleic acid isomers in the same maximum value of 0.01% to 0.02.

Analysis the value for the three families of fatty acids Saturated Fatty Acids (SFA), Mono unsaturated Fatty Acids (MUFA), and Polyunsaturated Fatty Acids (PUFA), results that olive oil from the Mallakastra Kalinjoti cultivar has high levels of MUFA (77.23) and PUFA (10.17) while Kalinjoti olive oil from Vlora has higher levels of SFA (14.4).

Table 4. Fatty acid composition of selected oil

Fatty acid	Kalinjoti (Mallakastra)	Kalinjoti (Vlora)
∑-TFA	0.051	0.048
∑-SFA*	12.58	14.4
∑-MUFA*	77.23	76.7
∑-PUFA*	10.17	8.9
MUFA/SFA	6.139	5.326
MUFA/PUFA	7.594	8.618
PUFA/SFA (P/S)	0.808	0.618
MUFA/SFA	6.139	5.326

The MUFAs are of great importance because of their high nutritional value and positive effect on oxidative stability of oils. Oleic acid (18:1), the predominant fatty acid in virgin olive oil, was between 73.65% and 76.15%. The oil from cultivar of Vlora had the lowest oleic acid and MUFAs levels compared to the other oil from cultivar of Mallakastra. Linoleic acid, which is much more susceptible to oxidation than MUFAs ranged from 8.15% to 9.31%. The linolenic acid level of Albania virgin olive oil samples was below the maximum value fixed by the IOOC (1.0%) and by the EU (0.9%) [IOOC, 2001; EC, 2003]. These findings are in agreement with the results found in various olive oil producing countries of the Mediterranean basin [Codex Alimentarius, 1981; Diraman et al. 2009; Ersoy *et al*. 2001; Gutierrez *et al*. 1999; Gurdeniz *et al*. 2006; Kamoun *et al*. 2007; Ollivier *et al*. 2003; Opoku – Boahen *et al*. 2012; Romero *et al*. 2003; Zengin *et al*. 2006].

The relationship between the saturated and polyunsaturated fatty acid content is expressed as P/S index. In the diet, it is important the index value be higher than 1, due to the essential character of the linoleic fatty acid (ω-6) (WHO, 1982). Several studies indicate that the P/S relation influences in the level of nutrient metabolization in the body, and as the proportion increases a smaller deposition of lipids (LAWTON et al., 2000). The values of the P/S indexes of the oil studied are shown in Table 4. As the result show the index P/S is lower than 1 in the both oils, because in olive oil consist small amounts of PUFA (Zambiazi et al. 2007).
The fatty acid composition of both olive oil groups from different locations of Albania has been shown to be similiar with regard to the ratios of oleic to linoleic acid and MUFAs to PUFAs which are an important indicator of oxidative stability (Table 3). The variations in fatty acid profiles of both olive oils differ depending on the zone of olive production. Primary factors affecting fatty acid composition, especially oleic acid content, in virgin olive oils may originate from latitude, climate, olive cultivar and/or stage of fruit maturity during harvest (Skevin *et al*.2003). Also, these values were similar to those reported for foreign olive oil varieties. All the values of fatty acids that were analysed, were in conformity to those of the IOOC's regulation (IOOC, 2001), the EU (EC, 2003). The level of palmitic acid (16:0), the major fraction of SFA in olive oil, ranged from 8.82% to 11.09% and the range of stearic acid levels (18:0) in samples were 1.61–4.25%. The little differentiation between structural isomers of these two fatty acids from the bothoils would bring a better knowledge of the chemical composition of olive oil and can be of great interest in their nutritional impact.
Fatty acid profiles played a role in the characterization of extra virgin olive oil from different geographical locations of Albania.

Conclusion

Basing on the results of the study, cultivar of Kalinjot presents qualitative and balanced profile of fatty acids, and trans – isomers, which the levels of the trans fatty acids are almost in conformity to those of International Olive Oil Council's regulation (IOOC, 2001) and Codex Alimentarius (1981), so we may conclude that production of the mono variety olive oil from Kalinjoti cultivar is in accordance with quality characteristics drawn by EU Regulation 1989/2003.

The fatty acid composition and the determination of trans fatty acid in the oil is a very important factor in the stability determination and main application definition. This factor is also associated to the harmful impact in the human health.

It represents an interest in producing a quality olive oil. Basing in the comparison with results from literature, it can be concluded that the levels of trans-fatty acids in the oils of Kalinjoti cultivar are almost similar to those in neighboring countries: Italy, Greece, and Spain.

According to the FFA and trans-isomers results, we conclude that the Mono cultivar Olive Oil, are classified as extra virgin olive oil (EVOO).

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DIFFERENCES IN COMPARED ACTIVITY OF WHITE FLIES PREDATORY POPULATIONS IN THE CITRUS REGION OF CHLEF (WESTERN NORTH OF ALGERIA)

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Abstract

This work is devoted for the first time to the study of the dynamics of predatory activity of the whiteflies populations *Aleurothrixus floccosus*Mask. and citrus whitefly *Dialeurodes citri* Ash. on clementine (var. clementine single) and orange (var. Thomson Navel) at the citrus region Chlef-Oued Sly (western north of Algeria). Populations sampling on the foliage at the young shoots bearing branches were conducted from the end of July 2012 to early July of the following year 2013. Predators as *Clitosthetus arcuatus Rossi*, *Semidalis aleyrodiformis Stephens* and *Chrysoperla carnea Stephens* were well represented, participating in the largest contribution differences in composition between the communities of seasonal beneficials. The temporal distributions of the adult abundances of *C. Arcuatus* were considered during the entire monitoring period for the cardinal directions (north, west, south and east), respectively. Adult abundances were characterized by 3 pics, a first one in early October, one second pic in mid-November and the 3rd largest in june with more than 300 individuals per yellow sticky trap per fortnight. During autumn period, the canopy foliage exposed to South and East is particularly sought after by adult lacewings *Chrysoperla carnea* with percentages of 42% and 33% respectively.

Key words: *citrus whiteflies, predatory populations, Chlef region, Clithostetus arcuatus, beneficial communities.*

Introduction

Whiteflies (Hemiptera: Homoptera: Aleyrodidae) are some of the most potentially detrimental pests for agricultural crops and ornamental plantings, (Byrne et *al.*, 1990; Martin 2004). Generally, aleyrodid populations are kept in check by natural beneficials. But, in agriculture crops where farmers have upset the natural balance, often substancial damaging populations may occur on the leaf undersides, due to producing waxy filaments and honeydew on which black sooty moulds grew. This interfered with the normal physiological activity of the host plant. The citrus whitefly *Dialeurodes citri* (Ashmead) and the woolly whitefly *Aleurothrixus floccosus* (Maskell) are one of the key pests affecting citrus in Algeria (Berkani, 1985; Benmessaoud-Boukhalfa,1987; EPPO, 2012).

However, their control is nowadays mostly chemical. Lots of predatory species, represented mainly by neuroptera and beetles help to control whitefly species. Natural biological control by lacewings is also among the most important integrated pest management (IPM) tactics for population regulation of the homoptera pests such as they have a high biotic potential, feeding on aphids, mealybugs, eggs, larvae and pupae of whiteflies occurring naturally on Clementine and orange citrus (Biche, 2012).

Because citriculture has a great economic importance in Algeria, more sustainable means of successful controlling Aleyrodid populations is needed. The tendency in biological control is to neglect the vast potential of indigenous predators, not only to reduce populations before they exceed the economical threshold but also to anticipate these beneficial arrivals in the

citrus groves (Mignon et *al.*, 2003; Gomez-Marco et *al*., 2015). Different inventories of predatory insects of citrus whiteflies and mostly of Homoptera pests affecting citrus has been realized in several citrus producing areas around the Mitidja region. Specifically, systematic and ecological studies on Coccinellidae predatory species found on citrus orchards have been published by Saharaoui and collaborators (2009, 2015). However, few works have addressed attention on these beneficial predatory families concerning their seasonal composition on citrus. Thus, our investigations have covered the producting zone of citrus in the chlef region, where studies on the biodiversity of natural enemies are globally poor documented in relation with populations of whiteflies trends and how they could be influenced by phytosanitary management, the host plant and climate.

Material and methods

The study was carried out between the end of July 2012 to early July 2013 on two conventional citrus orchards situated in the Chlef region (Algeria). The Chlef plain is the second extensive sublittoral plain of citrus plantings after the Mitidja plain with more than 5 709,50 ha of superficy. The 4 ha (a *Citrus sinensis* old orchard) and 2.34 Ha (a *Citrus clementina* old orchard) studied sites are located respectively at Oued Sly at a distance of 12 km from Chlef with a latitude of 36°05'53.15"N, Longitude of 1°13'02.11"E, and at 4 km from Chlef with a latitude of 36°10'04.56"N ; Longitude of 1°17'18.09"E.

The Aleyrodidae populations and associated predatory beneficial were determined on the foliage at the young shoots bearing branches and from yellow sticky plastic vertical traps. In each orchard, three traps were randomly and monthly sampled in the winter season. Every fortnight at the other seasons, we carefully examined one twig with leaves from cardinal directions through the canopy of ten randomly sampled trees. The significance of the differences in beneficial diversity between two seasons in one considered orchard was calculated by a resampling procedure (bootstrap) (Poole, 1974).

We tested the total similarities between seasonal beneficials by an ANOSIM (Analysis Of SIMilarity) using the index of Bray-Curtis as a measure of similarity and the P-values Bonferroni corrected given by 10 000 permutations. To assess which taxa are primarily responsible for an observed difference between groups (Clarke 1993), we used the program SIMPER (SIMilarity PERcentage) to calculate the contribution (in percentage) of each insect species to the differences observed between seasonal communities. We have pooled all samples to perform one overall multi-group SIMPER. All the multivariate analyses were conducted with PAST 1.98.

Results and discussion

Abundance variations in *A. floccosus* and *D. citri* populations with associated predatory species in the studied sites

Variations in abundances of the two whiteflies studies species on twigs with infested leaves and on traps, are shown in figure 1 and figure 2. The main predatory fluctuations species are different from an orchard to another. The seasonal trend along the studied period was similar for the both sampling types according to the two white flies species (figure 1 et 2).

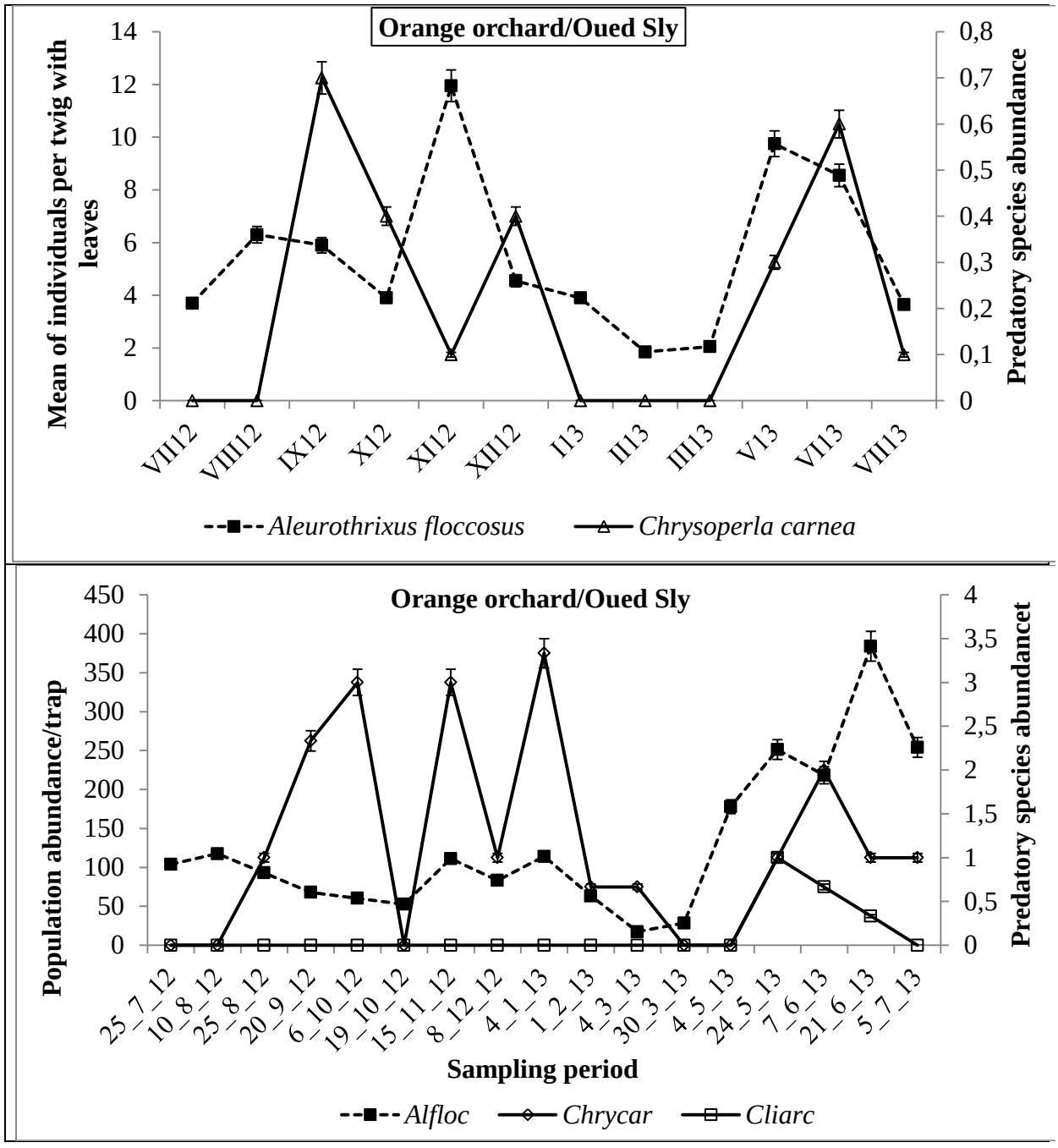

Figure 1: Seasonal abundances of *A. floccosus* and associated predators in orange orchard at Oued Sly (Chlef region)

Predators were well represented in this case *Clitosthetus arcuatus Rossi*, *Semidalis aleyrodiformis Stephens* and *Chrysoperla carnea Stephens* participating in the largest contribution differences in composition between the communities of seasonal beneficials.
Adult abundances of *C. arcuatus* were characterized by 3 pics, a first one in early October, one second pic in mid-November and the 3rd largest in june with more than 300 individuals per yellow sticky trap per fortnight. During autumn period, the canopy foliage exposed to the South and East direction is particularly sought after by adult lacewings *Chrysoperla carnea* with percentages of 42% and 33% respectively.

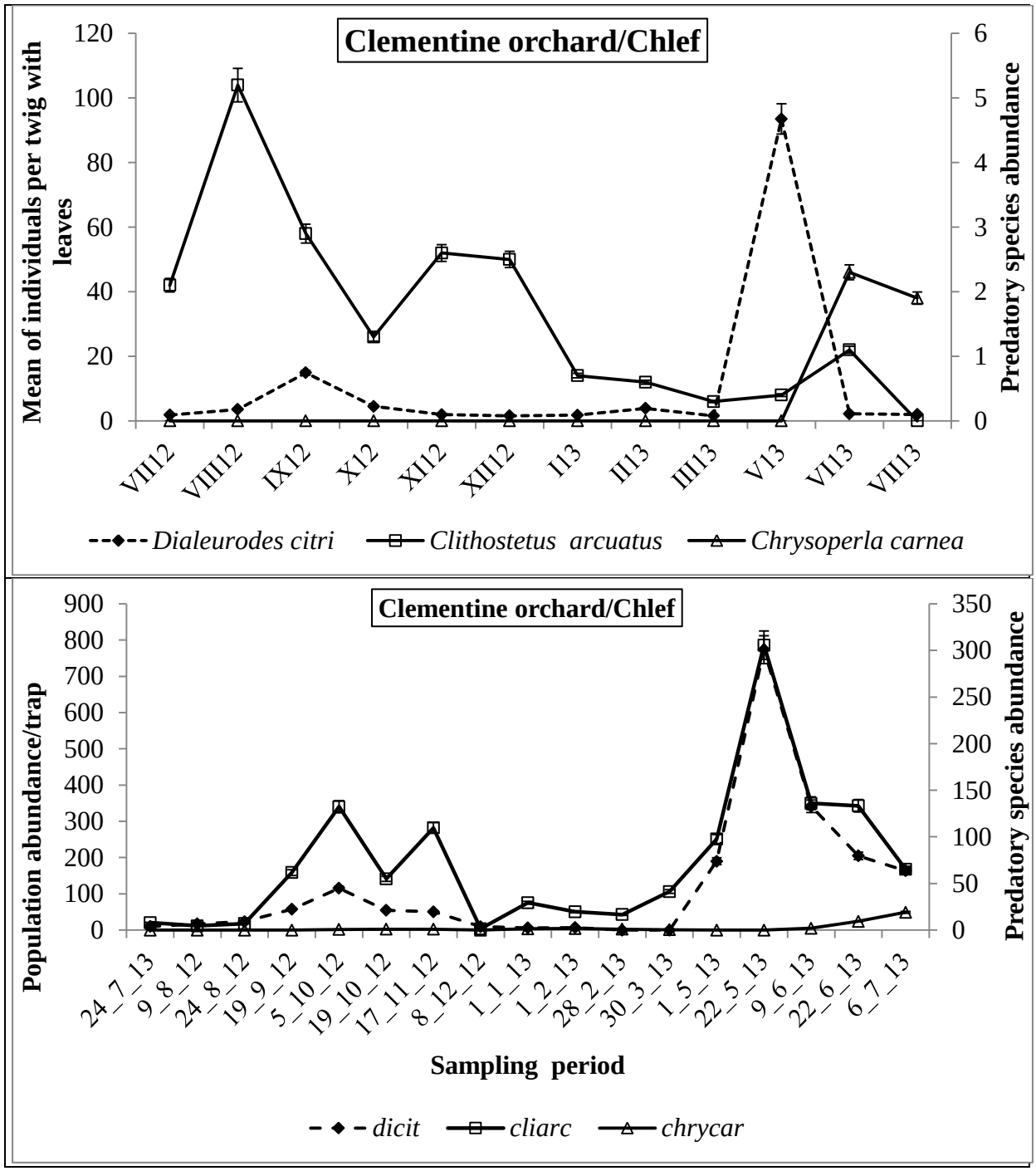

Figure 2: Seasonal abundances of *D. citri* and associated predators in clementine orchard at Chlef.

These observed results reflect possibly the abundance of preferred prey. Differences in incidence could be influenced by host preys and microclimate of the vegetation cover in the orchards wich could explain the coincidence and temporal arrival in relation with white flies populations.

Associated assemblages tendency

Semidalis aleurodiformis (Neuroptera, Coniopterygidae) was by far the most common species found among the identified predatory insects (table 1). These insects are very common on the citrus canopy. It is known moreover that coniopterygids are specially attracted to yellow sticky traps (Bru and Garcia Mari, 2003). *Clitostethus arcuatus* seems to be the most important predator of *D. citri*, showing a high voracity on the immature stages.

Table 1: SIMPER analysis of the seasonal assemblages of beneficials associated with *Dialeurodes citri* populations in orange orchard (2012-2013). (Numbers in bold characters correspond to the characteristic species of each community, LS12: late summer, AT12 : autumn, SP13 : spring, ES : early summer13).

Taxa	Contrib. %	LS12	AT12	SP13	ES13
Clitosthetus arcuatus	29,12	**2,33**	**11,5**	**56,9**	**20,8**
Semidalis aleyrodiformis	12,41	0	**25,5**	**6,4**	**9,5**
Aphidoletes aphidimyza	8,023	0	0,708	**13,5**	1
Braconidae	7,513	0	1,5	**9,8**	**9,83**
Eulophidae	2,798	0,667	**3**	0,4	**2,67**
Citrostichus phyllicnistoïdes	2,087	0	**2,87**	0,2	0
Scymnus subvillosus	1,866	**1,33**	**2,88**	0,867	0,333
Platigastridae	1,744	**2,08**	2,13	0,2	0,167
Ceraphronidae	1,63	**2,83**	0,25	0	0
Notochrysa capitata	1,618	0	**2,75**	0	0,167
Chrysoperla carnea	1,564	0	**1,5**	0	**2,33**
Conwwentia psociformis	1,384	0	**2,92**	**1,07**	0
Cynipidae	0,8989	0	0,833	0,8	0,333
Aphytis Chrysomphali	0,5488	0	0,75	0	0
Stethorus punctillum	0,5162	0,75	0,25	0,267	0
Aphytis melinus	0,4917	0	0,792	0,2	0
Encarsia sp	0,4621	0,167	1,04	0,333	0
Rodolia cardinalis	0,4495	0	0,333	0,267	0,667
Cales noacki	0,4256	0,5	0,417	0	0,167
Metaphycus flavus	0,4154	0	0	0,2	1
Megaspilidae	0,3935	0,167	0,125	0,667	0,167
Lysiphlebus testaceipes	0,3364	0	0,25	0,533	0
Pteromalidae	0,3108	0	0,25	0,133	0,333
Pachyneuronsp	0,2953	0,25	0,25	0,133	0
Mymaridae	0,265	0,167	0,417	0,2	0,167
Coccophagus lycimnia	0,2417	0	0	0,333	0,333
Anagrus atomus	0,2291	0	0,542	0	0
Gonatocerus sp	0,196	0,333	0	0,0667	0
Encyrtidae	0,1808	0	0,0833	0,267	0
Aphelinus mali	0,1362	0	0	0,267	0
Leptomastix sp	0,1157	0,167	0,0833	0	0
Trichogrammatidae	0,1123	0	0,208	0	0
Eretmocerussp	0,06957	0	0,0833	0	0

Table 2: Comparison between seasonal beneficial faunas in orange orchard (numbers in bold characters show the significant P values).

	latesumm12	autum12	spring13	earlysumm13
latesumm12	-	0,1921	**0,0467**	0,3298
autum12	-	-	**0,0077**	0,744
spring13	-	-	-	0,239
earlysumm13	-	-	-	-

The dustywings Coniopterygidae belong to the order of net-winged insects Neuroptera with some 6010 species. The family Coniopterygidae alone comprises about 450 species gathered in 23 genera (Aspöck et *al.*, 2001). The family is distributed in Europe, Africa, the Canary Islands, Asia, the Orient, and Australia, and also reaches North and Central America. Due to their very small size, many dustywing species may still remain undetected in Algerian citrus orchards. Among the aphid enemies, the parasitoïds (Hymenoptera) are often the first to establish themselves, followed by ladybirds then Anthocoridae, Chrysopidae intervene once the populations of aphids are in decline, (Saharaoui et *al.*, 2015).

The most important contributions to controlling *A. floccosus* have been through biological control programmes. In several Mediterranean countries where the pest was introduced, *Cales noacki* was better able than *Amitus spiniferus*, two Hymenoptera parasitoïd species, to reach high parasitization levels on the whitefly nymphs everywhere (Berkani, 1985). In citrus groves where chemical control was not applied, *C. noacki* was found to parasitize 99% of *A. floccosus* young stages, thus depressing the whitefly populations to very low levels throughout the year, in such a way that high infestations of the whitefly may occur towards late summer-early autumn because *C. noacki* development may slow down with the high temperatures and low relative humilities of summer.

Conclusion

The results obtained in this study highlight a seasonal variation of beneficial communities on citrus orchards in Chlef region. *Clithostethus arcuatus* was the most represented as the only specific aleurodiphagous species on white flies populations. Generalist predators seem to have a better activity during the hole occurring of the aleurodids prey populations especially *Semidalis aleyrodiformis* and *Chrysoperla carnea.*

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THE APHIDS INFESTING CITRUS ORCHARDS AND THEIR NATURAL ENEMIES IN THE NORTHWESTERN ALGERIA

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Abstract

During the last decade, especially in the Northwest part of Algeria, the citrus orchards showed fluctuations in production with a reduction of citrus growing areas in comparison with others growing areas. The decline of quality and quantity of production is a result of several problems occurring in the same time: aging of orchards, water stress, inadequate methods of control, emergence of new pests and diseases. The objective of this study was to determine the main aphid species causing serious damages directly and indirectly on *Citrus sinensis* and *Citrus clementina* and to identify their main natural enemies including parasitoids and predators. After two years of investigations the main aphid species recorded on both citrus orchards were: *Aphis gossypii* Glover, *Aphis spiraecola* Patch and *Toxoptera aurantii* Boyer de Fonscolombe (Hemiptera: Aphididae), *Aphis craccivora* Koch.The primary parasitoids recorded were mainly: *Lysiphlebus fabarum* (Marshall), *Lysiphlebus confusus* (Trembley and Eady), *Lysiphlebus testaceipes* Cresson, *Aphidius colemani* Viereck, *Aphidius matricariae* Haliday an *Binodoxys angelica*e Haliday (Hymenoptera: Braconidae : Aphidiinae).We also recorded an important activity of predators mainly *Propylea 14- punctata* (L.), *Coccinella septempunctata* (L.), *Scymnus* species (Coleoptera: Coccinellidae) , *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and 2 Syrphidae species but their presence happened too late. This study was conducted for a better knowledge on the health status of citrus orchards because the complex of aphid antagonists is poorly investigated in our region. This work could help citrus growers in understanding the situation for taking the best measures to preserve activity of natural enemies by reducing chemical control.

Key words: *Citrus, aphids, natural enemies, integrated control, Northwestern Algeria*

Introduction

Algerian citrus orchards harbour an important diversity of native and alien species of pests that attack trees in the same time and also a wide range of native and exotic natural enemies. Among the main pests damaging citrus groves, aphids are particularly noxious because high aphid population is regularly seen during spring season (during flushes) leading to reduction in vigor of the host plant and causing great damage both directly and indirectly (leaf deformation, malformation of flowers, underdeveloped shoots, virus transmission and consequently damage on fruits). In all citrus growing areas in Mediterranean Basin several aphids are considered harmful pests, (Barbagallo et al., 1986; Marroquin et al., 2004). In Algeria, studies conducted on aphids were concentrated in the Mitidja region (the north-center of Algeria).The number of species is depending on region, season, variety and period of investigation (Aroun, 1985; Mostafaoui et al., 2014; at least 08 species were recorded on *Citrus sinensis* var.Thomson (Benoufella-Kitous et al., 2014). In Greece 09 aphid species were reported on citrus (Kavallieratos et al., 2002) and in Turkey 07 species were determined (Satar et al., 2014). At least 25 aphid species have been reported infesting citrus in the world but only 3 or 4 species are considered damaging in Italy (Barbagallo et Patti, 1986) or in Spain (Hermoso de Mendoza et al., 1997) where *Aphis spiraecola* and *Aphis gossypii* are found to be the most abundant (Tena et Garcia-Mari, 2011). In the north-western Algeria

studies on aphids are very limited and the complex of their natural enemies very poorly investigated. The present work is a first step of a wide study undertaken by the first author within the framework of a thesis with several objectives in order to found appropriate measures for integrated pest Management. This paper lists the aphid species found infesting tender shoots during spring of 2014-2015 and makes inventory of natural enemies (parasitoids and predators) found feeding on aphids infesting new shoots at flush periods.

Material and Methods

Sampling method:

Aphids: During early spring aphid populations were monitored weekly on 10 trees, using only leaf count method. Samples were taken in a more 30 years old orchard with mixed varieties (*Citrus sinensis*, *Citrus clementina*). Three infested leaves were taken on young shoots from different levels of the canopy of each sampled tree and transferred to laboratory at (25°C, RH 60±10%, DL: 16H/24). In the laboratory live aphids are observed under stereo-microscope at adult stage (wingless and winged specimen) to identify the species according to several keys (when needed French or Italian specialists are asked for confirmation).

Natural enemies:

Parasitoids: In the laboratory, full mummies were taken from leaf with precaution and each one was put in individual small transparent box until emergence of wasps; empty mummies were counted as primary parasitoids when emerging hole is smooth or as hyper-parasitoids when the margin of emerging hole is irregular and added to total parasitism with identification of parasitized aphid species. Leaves bearing aphid colonies were placed with watered mesh in small boxes and left at least a week period at laboratory conditions to obtain new mummies from parasitized aphids. Total parasitism was estimated by counting emerged and none emerged aphid mummies.

Predators: Predators were separated according to their mobility using a smooth brush or mouth aspirator and put in small box with additional colonies of aphids until reaching adulthood stage. Specimens were conserved for later identification.

Results and Discussion

Aphids: In the first step of study only six aphid species were recorded. Aphid diversity showed 3 most important species found in all samples: *Aphis gossypii* (Glover) (fig.1 a,b), *Aphis spiraecola* (Patch.) (Fig.2) and *Toxoptera aurantii* (Boyer de Fonscolombe) (fig.3). Three other species: *Aphis craccivora* (Koch), *Myzus persicae* (Sulzer) and *Aphis fabae* (Scopoli) were found in limited colonies.

A. gossypii is a cosmopolitan, polyphagous species widely distributed in the world. It is one of the most pests of citrus in many Mediterranean countries (Kavallieratos et al. 2002; Satar et al., 2014). According to Vanlerberche-Massuti and Chavigny (1998) there is a genetic structuring within the species *A.gossypii* according to host plant type. This species cause direct damage by feeding on tender shoots and on flowers (fig.1b) and indirect damage by transmitting the Citrus tristeza Virus in citrus (Marroquín et al., 2004; Compra et al., 2000).

A. spiraecola is a polyphaous species found on *Citrus* species but it can infest other fruit trees of *Prunus* species in many Mediterranean countries (Ben Halima-Kamel et Ben-Hamouda , 2005). It is a key pest on *C. clementina* in Spain (Gomez Marco, 2015), in Algeria

(Mostefaoui et al., 2014) were compared to *A. gossypii*, its abundance on Clementine variety could be explained by a better tolerance to high proline contents in the foliage.

Toxoptera aurantii named Black Citrus Aphid is a pan- tropical species common on citrus trees. II is also found on tea and on other plants as *Pittosporum tobira* in the Mediterranean areas (Sahraoui and Sourreau, 2000). The parasitoid complex of *T. aurantii* in the Mediterranean area was reported in Italy (Tremblay, 1984) .The most important of its parasitoids are species of the genus *Aphidius* Nees, *Lysiphlebus* Förster.

Fig.1: *A. gossypii* on bud (a) and flower (b)

Fig.2: *A. spiraecola*

Fig.3: *T. aurantii*

N.B.: The aphid *Toxoptera citricidus* that was expected to be present in Algeria was not found in our samples. This species was recorded in almost all Mediterranean countries in Spain (Hermenzo de Mendoza et al., 2008), in Italy (Coccusa and Barbagallo, 2011).

Natural enemies

Predators: The most important families were Coccinellidae and Cecidomyiidae.

Fig.4: *Aphidoletes aphidimyza*

Fig.5: *Scymnus* spp. larva

Fig.6: *Coccinella algerica*

Fig.7: *Propylea 14punctata*

Coccinellidae: Although aphid-feeding coccinellids rarely play a role in the long-term regulation of population dynamics of aphid species within agro-ecosystems, they are effective predators reducing within-season densities of selected species of aphis pests (Obrycki et al., 2009).The Coccinellidae found to feed on aphids were *Coccinella algerica* Kovar, *Propylea 14 punctata* L., *Symnus* spp.

Cecidomyiidae: *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) (fig.4) is a worldwide predator used with success in biological control. It was found throughout Mediterranean citrus (Hermoso de Mendoza et al., 2012; Vacante et al., 2012; Urbaneja et al., 2014). Based on direct observations, larvae were first found in spring in all dense colonies of aphids killing more aphids than needed for their development. Similar observations were reported in Spain by Hermoso de Mendoza et al. (2012) with additional information concerning the number of potential generations per year.

Parasitoids: Primary parasitoids recorded belong to Aphidiinae subfamily.
The main parasitoids were: *Lysiphlebus testaceipes* (Cresson) (Fig.9), *Lysiphlebus fabarum* (Marshall) (Fig.10), *Aphidius colemani* (Viereck) (Fig.11)




Fig.8: Emerging parasitoid Fig.9: *L. testaceipes* Fig.10: *L. fabarum* Fig.11: *A.colemani*

L. testaceipes was recorded on *A. gossypii*, *T.aurantii* and *A.spiraecola.* According to Stary et al., (1988) this species was introduced from Cuba to Europe in 1973 in the Mediterranean part of France to control *T. aurantii* and *A. spiraecola.* It had established itself over the whole Mediterranean area where it was recorded not only in Europe but also in Africa; it was reported in 2010 in the South-eastern Algeria (Laamari and Coeur d'Acier, 2010) but its first record in Algeria dates from 1990 (Guenaoui,1990). Because this species is very invasive (the reason why it was removed from the positive list of biological control of EPPO) it is invading the continental part of Europe with a first detection in Serbia (Zikic et al., 2015).

L. fabarum was also recorded on *A. gossypii*, *A. spiraecola* and *T. aurantii*. Only bisexual strain was found in this study but an asexual strain was reported on *A. gossypii* for the first time in Algeria in 1993 at Mostaganem, (Guenaoui et Mahiout, 1993).This parasitoid is a koinobiont endoparasitoid associated with a wide range of aphid hosts in the world (Stary, 1988). In Algeria the sexual strain was found on 9 aphid species associated to 18 host plant species (Laamari et al., 2011). In Iran 47 aphid species were reported to be parasitized by this species (Rakhshani et al., 2013). *L. fabarum* plays a dominant role in several orchards. Parasitoid wasps belonging to the genus *Lysiphlebu*s (Foerter) are composed of over 20 species exploiting over one hundred species of aphids. In a recent study on genetic and morphological variation in sexual and asexual parasitoids of the *Lysiphlebu*s genus two groups were determined: the *fabarum* group and the *testaceipes* group with variation in morphs and reproductive mode, (Petrovic et al., 2015). In the *L. fabarum* species there is difference in trait life between sexual and asexual strains (Ameri et al., 2015).

A. colemani was recorded only on *A.gossypii.* This species was already recorded in Mostaganem (Algeria) on *Melanaphis donacis* on *M. persicae* and *A. gossypii* (Guenaoui, 1991) and on *Hyalopterus pruni* (Geoffroy) on pomegranate trees (Guenaoui, 1998). In the South-East Algeria this species was recorded on 47 aphid species infesting 85 host-plant species (Laamari et al., 2011).

Binodoxys angelicae (Haliday): Only one specimen of was found on *A. spiraecola.*

The richness of natural enemies is depending on the resources in the environment of citrus tree. For instance, in Italy as in Spain the role of weeds in the Integrated Pest Management of citrus was demonstrated (Ali-Arous, 2008; Urbaneja et al., 2014). In Spain the two first species were also found being the most abundant in citrus with 82% of *A. spiraecola* on *C. clementina.*

Conclusion

In the first step of study, the most abundant aphid species are *A. gossypii* and *A.spiraecola* followed by *T.aurantii*. Natural enemies were composed at least of 6 primary parasitoids with 2 main species (*L. fabarum, L. testaceipes*).The parasitism rate was low (below 5%) at the beginning with an increasing rate to15% by counting mummies. The mortality due to the parasitism before mummification was not evaluated. The activity of predators occurred too late with predominance of Coccinellidae (*P.14 punctata*, *C. algerica*) and Cecidomyiidae. The midge *A. Aphidimiza* showed a great efficacy in dense colonies but its action occurs too late

with a specific behavior called over-killing. In a future research, we need to conduct investigations to record more species and determine factors affecting colonization and abundance of aphids with evaluation of the incidence of intensive control of spontaneous vegetation, on aphids and on their natural enemies because growers are in the habit to destroy all growing weeds around trees.

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DENSITY INFESTATIONS OF PARLATORIA ZIZIPHII (LUCAS) (HEMIPTERA: DIASPIDIDAE) ON CITRUS IN THE NORTHWESTERN ALGERIA.

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Abstract

In Mostaganem area (Northwestern Algeria), a study on scales infesting citrus conducted during three years (1996-98) showed a predominance of *Parlatoria ziziphii* (Lucas) (Hemiptera: Diaspididae) with the hypothesis of destruction of its natural enemies by overuse of pesticides. In recent years, populations of this species increased dramatically leading to heavy damage on fruits that become unmarketable. The main objective of the present work was to analyze the role of different biotic and abiotic factors on seasonal abundance of scales infesting fruits. The present results refer only on the first year period of a wide study conducted in two sites on two varieties (*Citrus sinensis* var. Thomson and *Citrus clementina* var. Clementina). One hundred fruits per variety were observed *in situ* from July to the beginning of harvest. The density of scales on fruits had increased, reaching a mean of 6 scales per fruit before harvest. The importance of infestations was depending on the position of fruit on tree. The fruits located in the center were significantly higher infested. We also recorded natural enemies associated with *P. ziziphii*. The parasitism was very low (about 0, 25%) in September and October, increasing to about 10% in November. Through a field investigation we tried to identify real factors limiting effectiveness of natural enemies.

Key words: *Parlatoria ziziphii, fruit, Citrus, density infestations, natural enemies.*

Introduction

In Algeria, citrus orchards are attacked by several native and invasive pests with predominance of the diaspididae family named armoured scale insects. The black scale *Parlatoria ziziphii* (Lucas) (Hemiptera: Diaspididae) is an important pest of *Citrus*, widely distributed, throughout the Mediterranean basin. The pest was recorded on several hosts, mostly on *Citrus* (Rutaceae) (Stathas et al. 2007). It attacks all aerial parts of citrus trees (shoots, branches, leaves and fruits) and the main impact of damaged fruits is responsible in reject from national and international markets. Causes leading to diaspidid problems in citrus include salinity, heat stress, pesticide use, fertilization and pruning. Heavy infestations lead crawlers (neonate larvae) to migrate from leaves to fruit in summer. *P.ziziphii* causes pronounced discoloration with attached insect, even if the fruits are cleaned at the packing station (Benassy, 1985). Trees over ten years old are particularly prone to attack.

In Algeria, this pest is covering a vast extension of citrus orchards in the center (Mitidja region, le most important citrus area with more 40% of total cultivation (Benguendouz et al., 2011). In the northwestern Algeria a study showed that almost *Citrus* species were heavily infested with a rate of scales varying from 80% to 99% (Alouach, 1998). *P. ziziphii* is considered as a hard pest to kill because it is very difficult to set up efficient control strategies (Praloran, 1971). Citrus growers use only pesticides that lead to recurrent infestations in a short time with use more pesticides because farmers rarely consider resistance of pest as part of problem. In Mediterranean basin the scale develops 3-4 overlapping generations/year (in Tunisia (Benassy, 1975); in Egypt (Salama et al., 1985). The present work is a part of a study undertaken in a framework of a thesis conducted by the first author, on the most noxious diaspididae species in the northwestern Algeria including dynamics of scales on leaves and

fruits with estimation of impact of natural enemies with/ without integration of wild vegetation and with / without pesticides treatment). The main objective of this paper was focused on estimation of density populations of *P.ziziphii* on fruits by following the populations from the first contamination of young fruits by crawler larvae until the harvest period in order to detect the best period for insecticide application. This could reduce the number of applications during the vegetative period. The trials were conducted in unsprayed orchards in order to evaluate the parasitism and record the main predators feeding on the diaspidid.

Material and methods

Presentation of the site of study

The present study was conducted in 2014 and 2015 in two private citrus orchards located in Misserghin province in the northwestern Algeria (Fig.1). In the past, this region had been one of the most important growing citrus areas especially of *C.clementina* variety; this hybrid variety was born in a farm located in the Misserghine vicinity.
The sampled trees are more 30 years old.

Fig.1: Algeria map: Site of study ◯

Fig.2: Location of the citrus orchards (Google map, 2014)

The observations were done on two varieties (*Citrus sinensis* and *Citrus clementina*) (Fig.2).

Sampling method: The 2 experimental orchards were sampled during the fruit season. On each sampling date, 10 fruits per tree (2 fruits from each cardinal position and 2 fruits inside the center) were marked and observed weekly. 10 trees per variety were sampled. Observations (*in situ*) with a magnifying glass were realized in order to count all scales infesting fruit from the first infestation by mobile larvae (crawlers) until harvest. A total of 200 fruits were weekly followed. Natural enemies feeding on scales were recorded. The global parasitism rate was evaluated.

Results and discussion

1- Percentage of fruits infested by *P.ziziphii* in both varieties

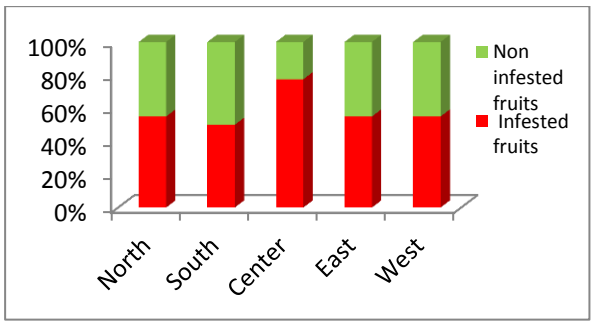


Fig.3: % of infested Clementina fruit (ICF)

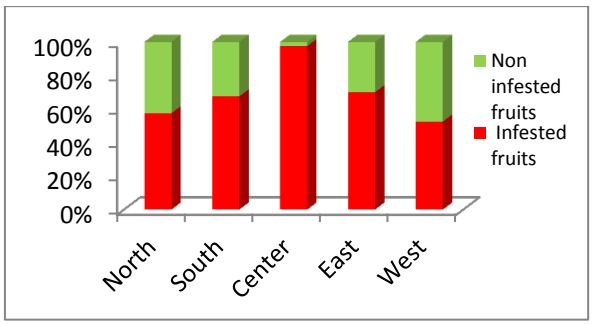


Fig.4: % of infested Thomson fruit (ITF)

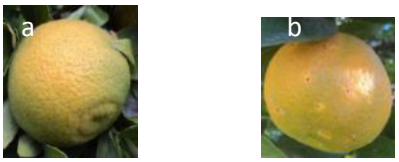


Fig.5: Non ICF (a); ICF (b)

Fig.6: Non ITF (a); ITF (b) (c)

2- Mean of scales per fruit:

To compare mean scale populations associated with each variety and orientation for each location means ANOVA test (Statbox 6,4) was used and means were separated using Newman–Keuls test (5%). The mean number of scales/fruit (Fig.7) increased from about 02 scales at the beginning (mid August) to 6 scales / fruit in late November on Clementina variety and from 1,6 scale to 5, 6 individuals on Thomson variety. There is no significant difference between varieties the ($p > 0, 05$). Regarding the position of fruit on the tree (Fig 12), the density population of scales per fruit is significantly higher in the center with 14,75 scales (Clementina) and 13,35 scales (Thomson).

Statistic analysis showed a significant difference of populations on fruit located in the center because there the diaspid *P.ziziiphii* is protected from wind and sun.

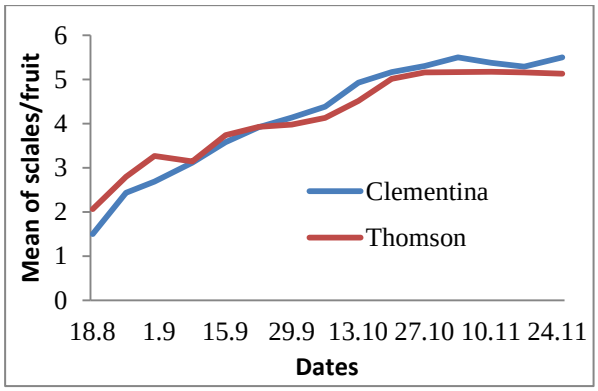


Fig.7: Mean of scales/ fruit

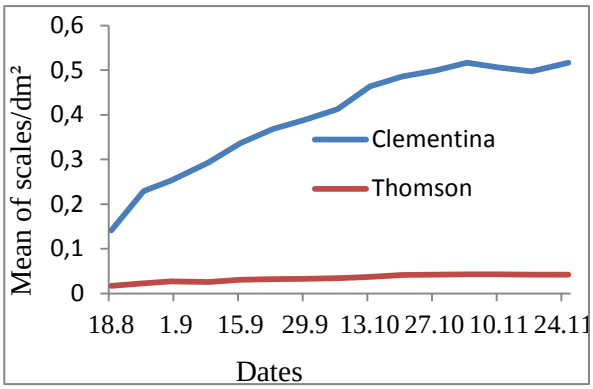


Fig. 8 : Mean of scale/dm² of fruit

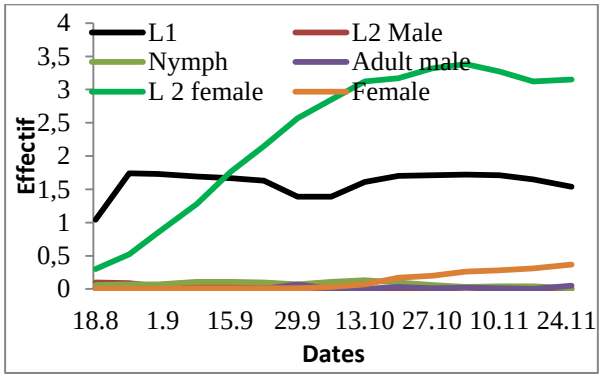


Fig.9: Number of scales/fruit/instar on Clementina variety

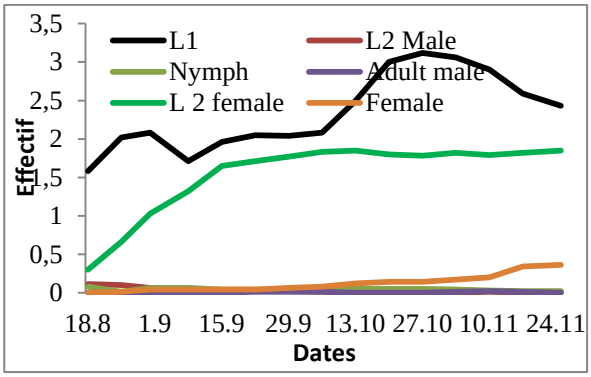
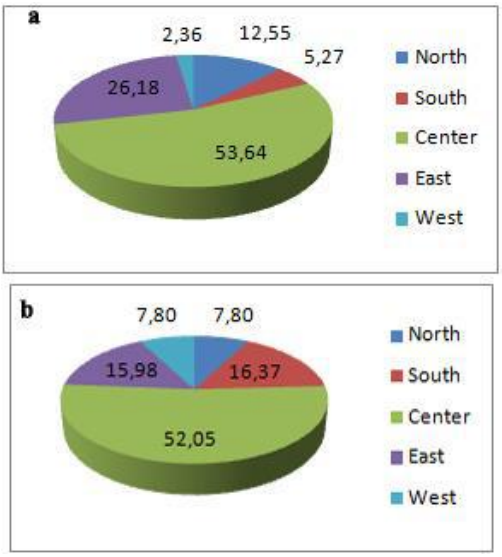


Fig.10: Number of scales/fruit/ instar on Thomson variety

Fig. 9 and Fig.10 show the distribution of young larvae and females + males adults.



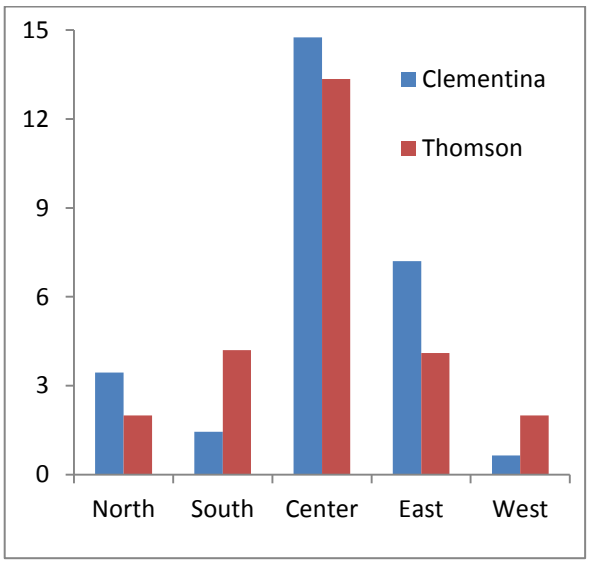
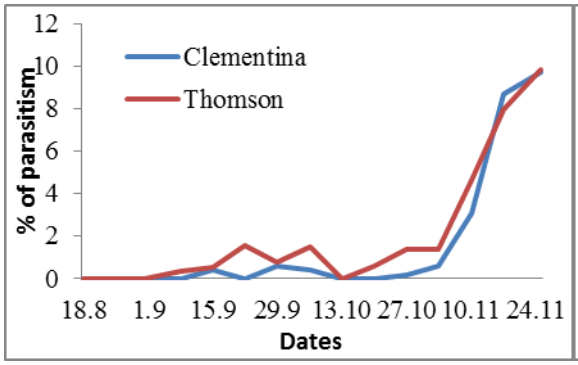


Fig.11: Rate of scales/ fruit on Clementina (a) and Thomson (b)

Fig.12: Mean of scales/fruit/orientation

The density populations of the diaspid on fruits are depending on variety, position of fruit and activity of natural enemies.



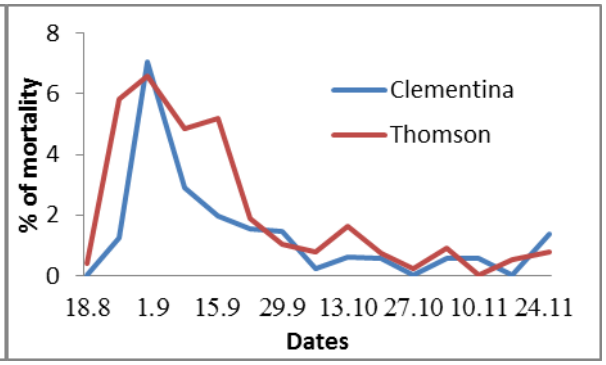


Fig.12: % of parasitism

Fig.13: % of mortality without parasitism

The total parasitism rate (primary parasitoids and hyper-parasitoids) is shown (Fig.12). It was below 5% until early October on both varieties and it increased to 10%. Use of chemicals could have a negative impact on the efficiency of natural enemies. The % of mortality was high in August and early September on the 2 varieties reaching 6-7% probably due to high temperatures and action of predators (Fig.14). In several cases, divergences between the thermal preferences of the host and those of the parasitoid lead to a disruption of the temporal or geographical synchronization increasing the risk of host outbreaks (Hance et al., 2007).

Fig.14: adult of *Chilochorus bipustulatus* (Coleoptera: Coccinellidae) on fruit.

Conclusion

Our field results showed that *P.ziziphii* is present throughout the year in all citrus trees. The leaves were heavily infested .The young larvae (crawlers) that are mobile moved from leaves to fruit. They insert their mouths and left their legs becoming fixed on fruits until death. The infestation density on fruit is depending on position of fruits on the tree. In the center the density was significantly higher because the scale is protected from sun and wind. In the center, pruning is not used in most of orchards. To control *P.ziziphii* on citrus we have to apply a strategy combining several measures using agronomic tactics such as pruning, in the center to prevent high infestations.lt will be important to spray (if needed) with efficient products when young larvae are settling on fruits.

Two *Aphytis* species were the main parasitoid of *P.ziziphii* but their efficacy is low. The most frequent predator feeding on fruit was *C. bipustulatus.*

In the current social context, the reduction of pesticides is strongly needed with the development of alternative solutions including biological control, agronomic measures.

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SEVEN YEARS OF STUDIES ON TUTA ABSOLUTA (MEYRICK) (LEPIDOPTERA: GELECHIIDAE) IN ALGERIA: WHAT HAVE WE LEARNED ABOUT?

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Abstract

This paper presents summarized a part of works conducted on *Tuta absoluta* since its first record in 2008 in Algeria. This invasive pest is always the most devastating tomato crop in the most important growing areas but main pests as aphids, whiteflies and mites are becoming problematic. Several studies on biological and ecological parameters, distribution, phytosanitary measures about *T.absoluta* were carried out during the last years. The main native antagonists (predators and parasitoids) were inventoried in different tomato growing areas in two far regions: In the vicinity of Mostaganem (Northwestern Algeria) and at The Oued- Righ Valley, a Saharian area located in the Southeastern Algeria. Natural enemies were sometimes misidentified. The effectiveness of some species is controversial. Tomato culture under unheated greenhouses is generally more confronted to attacks of *T.absoluta* than in open fields. Usually, farmers adopt chemical control and it is not easy to break them of this habit. To our great surprise, a prospection in more fifty small farms in the South part of Algeria revealed interesting findings: when tomato is cultivated in intercropping with aromatics plants or vegetables in association with date palm trees, attacks of *T. absoluta* were often very limited leading to suppression of chemical control. By these farming practices agricultural measures providing a good level of preservation of biodiversity are suggested. This study revealed a lack of knowledge about pest management practices, depending on season, on region and level of knowledge or standard of living of farmers. This paper explores and analyses the current limiting factors in the use of alternative measures such as biological control.

Keywords: *Tomato, Tuta absoluta, biological control, biological parameters, farming practices.*

Introduction

The invasive leaf-miner *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) originated from South America has become one of the most devastating pests of tomato crops over the world. Since its first record in Spain in 2006 (Urbaneja et al., 2007; Garcia-Mari et Vercher, 2010) the pest spread quickly over all the Mediterranean countries because it found there all suitable conditions. Year after year, new territories became invaded and the pest is continuing to spread throughout three continents (Europe, Africa and Asia). Recently, it has been reported for the first time in Maharashtra (India) in November 2014 (Sridhar et al., 2014).This biological invasion may be defined as a natural disaster because it is causing a rapid and damaging socioeconomic impact with unwanted consequences in many countries (Riccardi et al., 2011). In Algeria, the first damaged tomato crops by were observed in spring 2008 in Mostaganem province (North-western Algeria) (Guenaoui, 2008). The pest spread to all tomato growing areas reaching Biskra (South Algeria) the most important greenhouse area (Allache, 2012) with environmental conditions highly favorable. Its control is still very difficult due to several well known factors (high reproduction potential, easy selection of resistant strains to applied insecticides) but difficulties are particularly due to a low knowledge level of many tomato growers who aren’t initiated to talk about the new

approaches associating food security, economic growth and social stability well known in developed countries.

After seven years of studies on different aspects of the problem, what have we learned about this pest? Are-we capable to control it by applying the same methods used in developed countries? Are-we ready to apply ecological measures and safety tactics by changing deeply our behavior? We try to comment some controversial information on biology, behavior, and suitable host-plants. The relationships between the tomato leaf-miner and its new environment through the factors interacting in the agro-ecosystem should be taken into count in development of IPM program for control of *T.absoluta* in Algerian conditions.

1-Biology, Ecology and behavioral activity of *T.absoluta*

Material and Methods

Insects and host- plants

A population of old larvae were recorded on infested tomato plants grown under plastic house at the farm of the university of Mostaganem and transferred to young caged tomato plants for having young adults that could assure rearing at different temperatures to perform experiments on development time, fecundity, longevity… (for more details see Guenaoui et Ghelamallah, (2008); Dehliz and Guenaoui (2015**).** Seedlings were regularly potted and kept healthy for experiments. To determine the reproduction type, virgin females were introduced in caged tomato plants under optimal conditions. The experiments were repeated many times during three consecutive years.

Results and discussion

The larvae attack all parts above the ground starting from seedling till fruiting stages causing serious damage when an adequate control is not operated. Determining biological parameters is critical regarding various habitat conditions. The development time and survival of each larva and the fecundity of females were noted daily. The development time is depending on the climatic conditions; for instance a generation (egg-adult) took about 3 weeks at 27 ° C and 27 days at 25°C (Guenaoui et Ghelamallah, 2008). Under optimal laboratory conditions (25°C±1 and RH= 70 ± 10% with a photoperiod of 16/8 h (L/D). Net reproductive rate (R_0), intrinsic rate of increase (R_m) mean generation time (T) and mean generation doubling time (DT) were respectively 22 females/female/generation, 0,05female/female/day, 27,7 days, and 06,3 days. In the northwestern part of Algeria, at least 10 overlapping generations per year were obtained. When females are mated and fed with rich food (solution of honey) they can lay eggs on any substratum such as inner surface of bottle. Mean fecundity was estimated to be 72, 5 ± 55 varying from 02 eggs to more 200 egg. Longevity of adults was estimated to be 11days (female) and 04days (male) without additional food. Biological parameters showed differences regarding other published data but cultivar may be considered as the main reason for these difference .In spite of repeated works in the world, the biology of this species is not yet known well, seeing recently that the pest will be able to reproduce by parthenogenesis in a few cases (Abbes and Chermiti, 2012; Capparros Medigo, et al., 2012). In all experiments the reproduction of *T. absoluta* necessitated mating of female. The possible parthenogenesis in *T. absoluta* mentioned by Capparos et al (2012) lead to ask two questions: Why and how the pest reproduce by parthenogenesis? Why in its origin area (according to our knowledge) never this mode of reproduction had been cited in the past? In a study on parthenogenesis, calling behavior and insect-released volatiles of a leaf-miner moth belonging to *Phyllonorycter* genius, the asexual reproduction was observed by Mozuratis et al., (2002), but in despite a

complete absence of males, the authors observed that reproducing females diurnally demonstrated the calling posture normally used for releasing signaling compounds.

2-Is sweet pepper a suitable host plant for *T.absoluta* ?

T.absoluta can attack several plant species belonging to Solanaceous family including weeds, vegetables. We tested only the most important vegetables (tomato, potato, egg-plant and sweet pepper) with the main objective to test the suitability of *Capsicum annuum* (sweet pepper) for *T. absoluta* because it is often listed among attacked plants.

Material and methods

Potted and caged sweet pepper plants were offered to ten freshly mated and fed females during three successive days. In a series of experiments we operated by comparing 2 combinations: (1) sweet pepper + tomato, (2) only sweet pepper.

Results and discussion

The best suitable host plant for *T. absoluta* was tomato. On sweet pepper we observed eggs without hatching; few hatched larvae burrowed leaves but died before reaching adulthood stage. This can explain the attractiveness of tomato for *T.absoluta.* Sweet pepper is not a suitable host plant for *T.absoluta*. This question had been well documented by Potting et al. (2013) especially in the erratum 6 because import restriction for both fresh tomatoes and sweet peppers from areas invaded by *T.absoluta* was applied in many countries. This observation is a useful indication on the suitability of the tomato plant through its volatile compounds which are used to identify the host plant. In many cases olfactory orientation by pests is guided by specific volatile blends released from their hosts (Dahlin et al. 2015).This information could make possible use of volatile interactions between neighboring plants which can lead to change in their volatile emissions. Tomato plant that coexist with other plants not attacked by *T.absoluta* may be less attractive for *T. absoluta* due to volatile interactions. These changes in host plant volatile profiles induced by interactions between undamaged plants could be used to manage *T. absoluta* populations in crops.

3-IPM Strategies of control: Biotechnical tactics; Biological control, chemical control

Several strategies are used to control *T.absoluta*: pest monitoring, agricultural practices, chemical control, management by using sex pheromones for mating disruption, or mass trapping and at a less extent biological control (Gabbara et Arno, 2010; Al- Jboory et al., 2012; Savino et al., 2012; Biondy, 2013; Zappala et al., (2013); Chailleux et al., 2014; Ghoneim, 2014; INPV, 2014). Chemical control is the most used method.

Material and methods

We have tested the role of both insect proof nets and sex pheromones trapping in unheated plastic houses, bio pesticides and inventoried natural enemies of *T.absoluta* from 2010 to 2014, (for more details see Guenaoui et al., (2011a); Guenaoui et al., (2014) and Dehliz et Guenaoui (2015).

Results and discussion

Studies showed the importance of sex pheromones in managing *T.absoluta* because we can detect the first flights of moths. Using trapping by sexual confusion was very useful in choice of type of control according to the system cropping. New bio pesticides were recommended to growers receiving an efficient training (Gasmi et Guenaoui, 2012). Recruitment of natural enemies by *T.absoluta* was widely reported in Guenaoui et al., (2013) and Dehliz et Guenaoui, (2015) but misidentification of native species (Gebiola et al., 2015) introduced confusion in some results. Conservative biological control (Guenaoui et al., 2014) and more biodiversity with use of intercropping as seen in the Oued Righ valley (Dehliz et Guenaoui

2015), could contribute to a better sustainability (fig. 2). In Algeria growers are facing several problems because greenhouses are not isolated; using insect-proof nets and trapping by sex pheromones could reduce damages until 50% (Guenaoui et al., 2011a). Good control can be obtained if the treatments are carried out in greenhouses with good isolation which prevent moth from entering. More difficulties can appear when the number of males caught increase or is not adequate with the rate of larval populations. This situation leads to use more pesticides and induces growers to have a false opinion in mind because they believe use of trapping could increase damages. Conservative biological control with native natural enemies such as predatory mirids well adapted to the climatic variation (Guenaoui et al., 2011b) development of intercropping or presence of weeds around tomato greenhouses or in open field will be preferred to increase biodiversity (Guenaoui et al., 2014) (Fig.1).

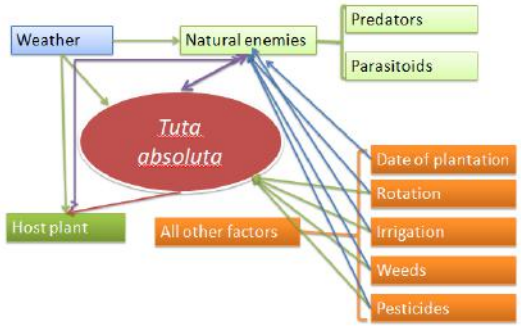

Fig.1: Interrelationships between the host plant/ *T.absoluta* and all other factors (biotic or abiotic)

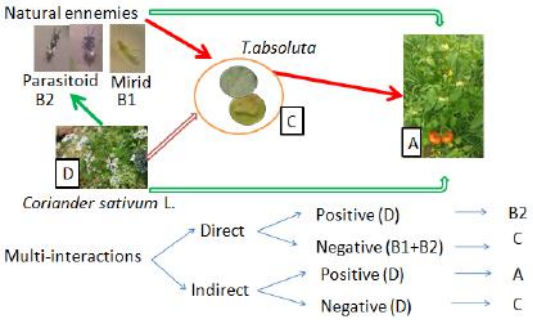

Fig.2: Interactions between *T. absoluta*, biocontrol plant and beneficials

General conclusion

In Algeria, since the occurrence of *T.absoluta* in 2008 biological and ecological studies have increased in number and sophistication over the types and severity of its impact on tomato production, economical and ecological aspects during the last seven years. The number of papers on the problematic *T.absoluta* is increasing but data on the effectiveness of different methods of control are diversely appreciated. In despite considerable support to tomato growers, many of them still face unacceptable losses. Adoption of Integrated Pest Management is slow. Studies have shown that several species of natural enemies associated with *T.absoluta* exist, showing that most parasitoid species are not efficient and may give useful control of this pest only in specific conditions.

It is so difficult to explain why and how to manage the pest when growers have not the adequate knowledge for understanding how to prevent attacks in order to reduce pesticides spays and favor natural enemies. If in developed countries (Europe) this period has created a much clearer picture of the biology and ecology with a increasing number of natural enemies species of *T. absoluta* that has led in the same time to increase studies for management studies, we believe without a clear understanding of the economic activities, it is impossible to explain and understand biology in a context where there is a lack of coordination between growers, managers, scientists, teachers and consumers. The insecticidal sprays are applied using high volumes that lead to more residues on marketable tomato fruits and less efficacy with promoting resistant populations strains as in Mediterranean countries. Integration of augmentative biological control with adequate releases of predatory mirids with other control methods could be complex but feasible however in Algeria this method has not been sufficiently explored to improve performance. Innovations are now costly; we really need a public understanding of the environmental consequences of insecticide alternatives for use of

biological control. We should increase efforts keeping in mind that the success of control of a pest depends also on the individual competence of farmer. A better understanding the interactions between human activities and natural environment integration is possible only when growers are able to take decisions towards more efficient and sustainable agricultural practices.

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PHYSICOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF DATES SYRUPS (Phoenix dactylifera L.)

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Abstract

Date fruits of date palm (*Phoenix dactylifera* L.), are among the most important agricultural products of Algeria. The biochemical composition of the two varieties of dates: Deglet-Nour and Mech-Degla, is marked by the high sugar content 70.92-74.07% fresh weight (fw). Due to its high level of sugar, the dates are suitable for production of syrup. The physicochemical characterization of dates syrups has revealed that this product has a Brix of 70.46-70.56%, total sugars (60.28-61.41% fw), sucrose (35.97-36.25% fw), reducing sugars (24.31-25.16% fw), pectin (0.74-1.43% fw) and ash (1.24-1.35% dry matter). Determination of minerals indicated that syrup is good source of many important elements such as potassium and magnesium. In addition, dates syrups are a good source of polyphenols, with a limit of 57.95-72.50 mg gallic acid equivalent (GAE) per 100g of fresh weight. Their antioxidant capacity ranged between 48.34-90.43% on DPPH assay, which leads to consider dates syrups as a functional food. Significant differences ($p<0.05$) were observed on pH, sugars, ash, potassium, calcium, copper, pectin, phenolics and antioxidant activity between the two dates syrups studied. Date fruits are products which could be processed into syrup with good nutritional values and broad prospects in food and pharmaceutical industries.

Keywords: date fruits, syrups, physicochemical composition, antioxidant activity, functional food.

Introduction

The date palm has been an important cultivar in arid and semi-arid regions of the world. It is principally cultivated in North of Africa and Middle East. Dates, the fruit of the date palm, is constituted the main food for the people of desert and its world production is estimated 7 127 952 in 2010 (FAO, 2012), more than 80% of this production comes from the Arab World (Al-Mamary et al., 2011). Algeria is the 4th largest producer of dates with a rich genetic heritage reaching 940 cultivars (Hannachi et al., 1998) and an annual production of about 789357 tons (FAO, 2012). Approximately, 48.21% of the annual production is made up of good varieties: Deglet-Nour (MADRA, 2010). The remaining production includes common dates, which are less appreciated by consumers and not valued especially dried varieties. Nancib et al. (1997), reported that 60000 tons of dates are lost every year in Algeria. The rejection of these dates is a real economical loss, because they are rich in noble components especially sugars, and as they may be transformed into products of high added value such as: jams, dates flour, syrup, vinegar, juices (Al-Farsi et al., 2007; Benamara et al., 2008; Al-Mamary et al., 2011). Date syrup is one of the products issued from dates processing, the well known, is prepared by a traditional process: concentration of an extract of dates by boiling, at atmospheric pressure. The date syrup is consumed either directly or can be used as an ingredient in the formulation of many foods, such as cakes, juices, confectionery, ice cream, yogurt and bread (Khalil et al.,

2002; Sidhu et al., 2003) and pharmaceuticals products like tablet binder (Alanazi et al., 2010).

The objective of this work is to investigate the possibility of the transformation of local dates varieties like Mech-Degla and Deglet-Nour to syrup in order to improve the traditional process using vacuum concentration and to preserve the nutritional and organoleptic syrup quality (color). In addition, this study evaluates the nutritional quality and antioxidant capacity of the elaborated dates syrups.

Material and methods

Raw materials

Two dried dates are selected for this study, namely Mech-Degla and Deglet-Nour. They come from the palm groves of the South-East of Algeria. They are harvested at full maturity and stored at 4 °C.

Syrups production

In the laboratory, dates are first cleaned and sorted out to remove soiled and molded fruits. Sorted dates are pitted and crushed, then mixed with distilled water at a ratio flesh/water: 1/3. The mixture is heated at 72°C for 30min with periodic stirring, then filtered through a sieve of 50 microns; the obtain extract dates is concentrated at 70 °C and under vacuum (410-430 mmHg).

Analysis Method

Fresh dates and syrups

The moisture content is determined by drying the sample of flesh dates in a vacuum oven at 70 ± 2°C. The pH was measured by a pH meter ADWA type, AD1030, at 20 °C (AFNOR, 1970). The soluble solids content is determined by measuring the Brix at 20 °C by using a Digital refractometer, Reichert type, AR 200. After clarification of aqueous extract of date, total sugars were determined by the method of Dubois et al. (1956). Reducing sugars were determined by the method dinitrosalycilic acid (DNS) (Miller, 1956). The sucrose content is deduced by the difference between the rate of total sugars and reducing sugars.

The percentage of purity is determined as follows (Mathlouthi and Reizer, 1995):

Purity = (Total sugars /TSS ratio) x100.

The ash is determined by official method of the AOAC (1995, No. 940.26). Minerals (K, Ca, Mg and Cu) were determined by atomic absorption spectrometry according to the AOAC standards (1995), using an atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 100).

Pectin was dosed in the form of aluminium pectate according of method described by De Luca and Joslyn (1957).

Polyphenols extraction

The extraction of phenolics from dates is made as follows: 1g of crushed dates was macerated in 40ml of methanol with continuous agitation for 24 hours. The mixture was filtered, and then the filtrate was evaporated using rotary evaporator (Heidolph type).

Total polyphenols determination

The total polyphenols were determined colorimetrically using Folin Ciocalteu reagent as described by Meda et al. (2006). The date's extract (500µl) was mixed with 2.5ml of Folin Ciocalteu reagent (0.2N). A 2 ml of Na_2CO_3 (7.5%) was added to the mixture, and then the mixture is incubated for two hours in the darkness. Read the absorbance at 760 nm. The total polyphenol content is expressed as gallic acid equivalents (GAE).

Determination of antioxidant activity by DPPH radical

To 250 µL of methanol extract, was successively added 01.75 ml of methanol and 0.5 ml of the solution of DPPH (0.2 mmol). The mixture was placed in darkness at room temperature for 15 min, then its absorbance was measured at 515 nm. A control sample, free from dates

extract was prepared under the same conditions. The inhibition of the oxidation of DPPH was calculated using the following equation:

Inhibition (%) = $[(A_0 – A_1)/A_0]$ x100

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Statistical Analysis

Results were expressed as mean ± standard deviation and analyzed by SPSS (version 20). Duncan's test ($p< 0.05$) was used to determine the significant differences between mean values. All analyses were conducted in triplicate.

Results and discussion

Physicochemical characterization of flesh dates

The results of the physicochemical date's composition are shown in table 1.

Table 1. Physicochemical characterization of dates varieties

Parameters	Mech-Degla	Deglet-Nour
Moisture (%) [1]	11.80 ± 0.24a	09.94 ± 0.55b
Total sugars (%)[1]	70.92 ± 1.55a	74.07 ± 01.47a
Reducing sugars (%) [1]	10.89 ± 0.33a	13.26 ± 0.01b
Sucrose[1]	60.03 ± 1.41a	60.80 ± 1.48a
Ash [2]	02.05 ± 0.11a	02.42 ± 0.12b

[1]: fresh weight.

[2]: dry weight.

Means in line with different letters are significantly different ($p< 0.05$).

The moisture content of dates varieties: Mech-Degla and Deglet-Nour are respectively 11.80 and 09.94 %, with a significant difference ($p< 0.05$). These values are similar to those cited by Al-Hooti et al. (2002), with contents of 11.53 and 11.55% respectively for the two Saudi varieties: Birhi and Safri. These dates could be classified as dry dates where the moisture content is less than 20% (Booij et al., 1992).

The studied dates were characterized by the predominance of sugars. The rate of total sugars is respectively 70.92 and 74.07% for Mech-Degla and Deglet-Nour dates of fresh weight (no significant difference).

It is well to note that the difference in the biochemical composition recorded between varieties of dates depends on several factors such as: variety, soil type, fertilization, irrigation conditions and climatic conditions (Booij et al., 1992, Al-Farsi et al., 2007).

Physicochemical characteristics of dates syrups

Table 2, shows the results of the physicochemical composition of dates syrups.

The moisture content of dates syrups Mech-Degla and Deglet-Nour, is respectively 24.64 and 25.49 %, with no significant difference ($p<0.05$). These values are relatively low and guarantee syrups conservation. From table 2, sugars are the major components of syrups, they contain a rate between 60.28 and 61.41% of fresh weight, representing 79.98 and 82.41% of the total dry matter respectively of Mech-Degla and Deglet-Nour syrups.

The reducing sugar content was recorded as 24.31 and 25.16 % for the date syrups of Mech-Degla and Deglet-Nour respectively, with a no significant difference. Whoever, acidity and thermal treatment applied during the concentration accelerated the conversion of sucrose into reducing sugar (Ben Thabet et al., 2009).

The date syrup is a good source of energy; it is rich in sugars, easily assimilated by the human body. In addition, the syrup could be used as a liquid sugar in many food products.

The purity of syrups is respectively 85.42 and 87.14% for the syrup of Mech-Degla and Deglet-Nour, with no significant difference ($p<0.05$). This purity is lower to that found by Mohamed and Ahmed (1981), which is 93.42% for the date syrup of Libya. The pH of

Deglet-Nour syrup (4.88) is more acidic than Mech-Degla (5.31), with a significant difference. The acidity of Deglet- Nour syrup is much higher than that of Mech-Degla. This is related to the acidity of the date used for the production of syrups (table 2).

Table 2. Biochemical characterization of dates syrups

Parameters	Mech-Degla syrup	Degle Deglet-Nour
Dry matter (%) [1]	75.36 ± 0.24a	74.51 ± 1.74a
pH	05.31 ± 0.01a	04.88 ± 0.01b
Brix (%)	70.56 ± 0.11a	70.46 ± 0.05a
Total sugars (%)[1]	60.28 ± 2.54a	61.41 ± 1.45b
Reducing sugars (%)[1]	24.31 ± 3.37a	25.16 ± 3.74a
Sucrose (%)[1]	35.97 ± 02.43a	36.25 ± 03.74a
Purity (%)	85.42 ± 3.73a	87.14 ± 1.99a
Ash (%)[2]	01.24 ± 0.03a	01.35 ± 0.05b
K[2]	512.63 ± 01.06a	634.27 ± 44.64b
Ca[2]	20.94 ± 0.26a	27.97 ± 0.02b
Cu[2]	0.84 ± 0.01a	01.53 ± 0.01b
Mg[2]	43.60 ± 2.15a	39.44 ± 0.07a
Pectin (%)[1]	01.43 ± 0.19a	0.74 ± 0.03b

[1]: Fresh weight.
[2]: Dry weight (mg per 100g).
Means in line with different letters are significantly different ($p < 0.05$).

Ash content of Mech-Degla syrup is 1.24%. This value is less than that of Deglet-Nour, which is 1.35% of dry weight, with a significant difference. These levels are comparable to those quoted by Al-Farsi et al. (2007), giving a percentage of 1.23 and 1.76% dry weight for the two dates syrups obtained from the Omani varieties: Shahal and Um-Sellah respectively. The ash content in the produced syrups dates is much higher (1.24-1.35% dry weight) compared to honey content, with an average value of 0.169% (Suarez et al., 2010). Indeed, sucrose contains minerals traces of potassium, calcium and magnesium with the following values 2.2, 0.6 and 0.2 mg per 100 g, respectively (Vierling, 2003).

The date syrup is a good source of minerals (table 2). Potassium is the dominant element of the mineral profile dates syrups. This has been reported by several studies (Khalil et al., 2002; Abbes et al., 2011). They contain 512.63-634.27 mg/100g of dry matter. Significant differences were also observed between levels of potassium, copper and calcium. The highest level of calcium and copper, are noted for Deglet-Nour syrup, on the other hand Mech-Degla syrup is higher in magnesium content (table 2).

The content of pectin found in date syrups is 1.43 and 0.74% respectively for the syrup of Mech-Degla and Deglet-Nour, with a significant difference. These values are comparable to those found by Alanazi et al. (2010), which is 1.46% for the date syrup Khalas (Saudi Arabia), but much higher than those reported by Abbes et al. (2011), which are 0.43 and 0.449% of fresh weight, respectively for dates syrups obtained from Tunisian varieties: Deglet-Nour and Kentichi. These differences appear to be due to varieties of dates. Pectin is soluble fiber. They have a beneficial effect on health, it improves intestinal transit and prevents against cancer. In the food industry, they are used as additives for gelling and stabilizing role in the production of jams, fruit juices, jellies and milk drinks.

Total phenolics and antioxidant activity

Results of the polyphenols content and antioxidant potential of dates syrups are presented in table 3.

Table 3. Polyphenols content and the reducing power of dates syrups

Parameters	Mech-Degla	Deglet-Nour syrup
Polyphenols [1]	57.95 ± 0.40a	72.50 ± 4.21b
Antioxydant activity [2]	48.34 ± 3.11a	90.43 ± 6.87b

[1]: mg of gallic acid equivalents (GAE) /100g.
[2]: on %.
Means in line with different letters are significantly different ($p < 0.05$).

The date syrup is a good source of natural antioxidants (polyphenols) and could be considered as a functional food (Abbes et al., 2011). A significant difference was observed between the rates of polyphenols dates syrups. They contain a 57.95 and 72.50mg of GAE/100g, respectively for Mech-Degla and Deglet-Nour syrup. In comparison, these levels are lower than those quoted by Al-Farsi et al. (2007), which gave values of 96, 141 and 162 mg of GAE/100g of fresh weight for syrups obtained respectively from varieties of Omani dates following: Shahal, Um Sellah and Mabseeli. This difference amounts recorded at polyphenols contents is due to the variety of dates used for the production of syrups.

The date syrup has significant antioxidant potential, which has been noted by several studies (Al-Farsi et al., 2007; Al-Mamary et al., 2011). The antioxidant activity was 48.34 and 90.43 % of DPPH scavenging activity, respectively for the syrups of Mech-Degla and Deglet-Nour, with a significant difference. A significant correlation was observed between the rate of total polyphenols and antioxidant activity ($r = 0.91$, $p < 0.05$).

Conclusion

According to this work, it is possible to develop this type of dates, in order to produce syrup from the perspective of date processing in the form of marketable products and high added value. The date syrup is a promising product that offers many advantages over sucrose syrup (refined white sugar): rich in certain nutrients such as minerals and other phytochemicals with health benefits such as pectin and polyphenols. The latter wich displays an interesting antioxidant activity. The date syrup provides unique functional and nutritional properties. It could be used in many foods preparations and therefore improves their nutritional quality and allows consumers to return to natural products. In the future, it will be interesting to determine the flavoring and rheological characteristics of syrups.

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SOME BIOCHEMICAL COMPOUNDS EVOLUTION IN SEVERAL CHICKPEA GENOTYPES CALLI, ELICITED WITH ASCOCHYTA RABIEI SPORES SUSPENSION

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Abstract

Chickpea *Cicer arietnum* is an important legume cultivated worldwide owing to its nutritional value, agronomic, ecological and economic importance. Its culture is exposed to various abiotic and biotic constraints, mainly ascochyta blight caused by fungus *Ascochyta rabiei*. When interacting with this parasite, the plant displays a cascade of defense mechanisms and undergoes changes in its metabolism. The purpose of this work is to follow some biochemical compounds evolution in different chickpea genotypes, ICC3996 C, INRA 199, ILC 200 and ILC483 inoculated with *Ascochyta rabiei*. A pathosystem including callus (host) and spores (pathogen) is used as a simple way to study this interaction *in vitro.* To obtain calluses, stems cut are cultivated on Murashige and Skoog (1962) medium added with ANA/ BAP plant growth regulators. They are then inoculated, harvested after 0, 5, 10, 15 and 20 days then analyzed. Soluble sugars total, proteins, polyphenols and flavonoids are extracted and quantified.

These markers can possibly serve as indicators in the selection of resistant genotypes.

Key words *Cicer arietinum*, callus, *Ascochyta rabiei , polyphenols, flavonoids*.

Introduction

Chickpea (*Cicerarietinum* L.) is an important grain legume cultivated on about 12 million ha worldwide with a yield of 8.28 million tons annually (Akidobe and Maredia, 2011). It offered high quality proteins, vitamins and minerals for human consumption especially in developing countries where the nutritional intake from animal sources is limited. It’s also a hopeful crop for alternative agriculture in industrialized countries due to its capacity to fix near 70% of atmospheric nitrogen mainly by its symbiotic relationship with *Rhizobiumciceri* (Zaman et *al*., 2010). Unfortunately and in spite of the large important needs, yield of chickpea has not significantly increased these last decades mostly in Mediterranean basin countries (Benzohra et *al*., 2010), due to several biotic and abiotic factors. This culture is mainly influenced by Ascochyta blight as a biotic stress. This interaction causesbiochemical metabolisms changes.

Biotechnology tools especially callogenesis provide a simple system to quantify some compounds, aim of this research. Calluses derived from chickpea host plant are interacted with pathogenic agent spore suspension. Thereafter, their biochemical profile is studied. This can provide explanation about chickpea resistance or susceptibility and promote this plant Ascochyta blight resistance screening.

Material and methods

Chickpea genotypes namely, ILC 200 and INRA 199, ICC 3996 C and ILC 483 were as calluses source, used as host (Fig 1).

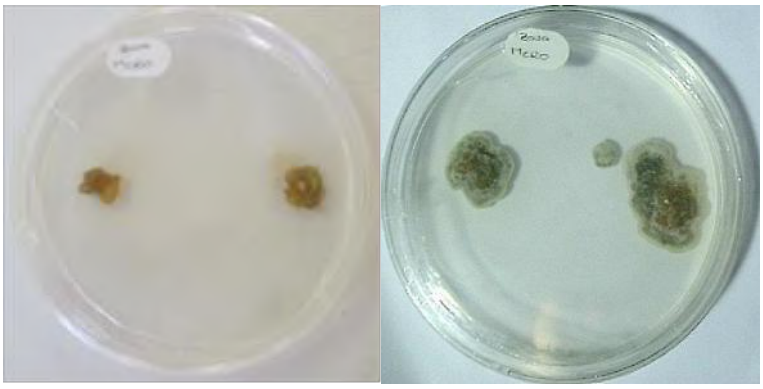

Figure 1 Pathosystem using Callus and spores suspension in early stage of interaction (a) and after one week (b)

Stem cuts were cultivated *in vitro* on Murashige and Skoog (1962) supplemented with 0.5mg/l NAA (1-Naphtyl acetic acid) and 1mg/l 6-benzylaminopurine (BAP). They were incubated under 22° ± 2C and 16h light/ 8h darkness photoperiod regime. Obtained calli were inoculated with a 10^6 *Ascochyta rabiei* spore suspension. Samples were harvested after 0, 5, 10, 15 and 20 days after inoculation to estimate biochemical compounds related to host and parasite interaction in chickpea Ascochyta blight. A methanolic extract is prepared for each genotype.

Soluble sugars were quantified following Dubois *et al*. (1956) method, total polyphenols with the Folin-Ciocalteu reagent method (Marigo, 1973), total proteins rate using Lowry et *al*. (1951) techniqueand flavonoids $AlCl_3$ essay(Quettier *et al*., 2000). A Completely Randomized experimental Design (CRD), with 3 replications was used. A multivariate analysis of variance (MANOVA) was used to study genotype, inoculation and interaction time effect on metabolites rates. The means comparison was done via Duncan's Multiple Range Test at 5% level.

Results and discussion

Generally, the results of MANOVA showed that genotype, inoculation and interaction time effects and their interaction were highly significant (P ≤0.001; Table 1).

Table1 Multivariate tests (MANOVA) results on biochemical compounds *Cicer arietinum*L. callus inoculated with *Ascochyta rabiei*

Effect		Value	F	ddl	Sig.
Genotype (G)	Pillai trace	3,687	141,253	20,000	**0,000 ***
Time (T)	Pillai trace	1,891	13,453	16,000	**0,000 ***
Treatment (Tr)	Pillai trace	0,707	34,446	4,000	**0,000 ***
G*T*Tr	Pillai trace	2,901	7,919	80,000	**0,000 ***

*Significant at α<0.05

Because the MANOVA technique just gives an overall test of the equality of mean vectors of several groups and in order to provide information on which variables are responsible for the differences in the mean, Duncan test were performed at a significance level of P ≤0.05 in the following analyses (Tab. 2).

Table 2 Intergroup effects on soluble proteins, sugars, polyphenols and flavonoids accumulation

Effect	Variable	Square sum type III	ddl	Square mean	F	P
Genotype (G)	Proteins	5,373	5	1,075	765,235	0,000 *
	Sugars	5,392	5	1,078	227,849	0,000 *
	polyphenols	0,119	5	0,024	570,766	0,000 *
	Flavonoids	25,973	5	5,195	953571,137	0,000 *
Time (T)	Proteins	0,142	4	0,036	25,361	0,000 *
	Sugars	0,337	4	0,084	17,807	0,000 *
	polyphenols	0,023	4	0,006	137,076	0,000 *
	Flavonoids	,003	4	0,001	154,104	0,000 *
Treatment (Tr)	Proteins	0,014	1	0,014	10,149	0,002 *
	Sugars	0,006	1	0,006	1,360	0,248
	polyphenols	0,006	1	0,006	140,403	0,000 *
	Flavonoids	0,00003	1	0,00003	0,707	0,404
G*T*Tr	Proteins	0,043	20	0,002	1,545	0,099 *
	Sugars	0,748	20	0,037	7,905	0,000 *
	polyphenols	0,022	20	0,001	25,833	0,000 *
	Flavonoids	0,006	20	0,000	53,476	0,000 *

*Significant at $\alpha<0.05$

Total Proteins

Proteins produced by host plant cells are determining to plant tissue defense against pathogen (Nawrot- Chorabik, 2013). Some of them may be enzymes involved in phytoalexin biosynthesis, or are related to plant defensins, while others are proteinase inhibitors that disrupt pathogen (Guest and Brown, 1997). For INRA 199 (Fig.2 c), inoculated showed a 23% increase in total proteins rate in the initial stage of infection and then decreased to 0.0273mg/ g fresh weight callus ± 0.0039. ILC 483 proteins levels were generally lower in inoculated calli compared to control. During the 5 first days, in both inoculated and control was relatively constant and no significative difference was observed. Five days post inoculation, proteins rates remained constant in inoculated calli, however, it showed a 50% increase for control and was significantly ($p<.0.05$) twice higher than inoculated one (Fig.2 d). For ICC3996 C no significant difference in proteins rates was observed during this experiment between control and inoculated calli (Fig. 2 a). In ILC 200 (fig. 2 b) case, generally proteins rates were significantly lower in inoculated callus compared to control especially in the 10^{th} day where were registered 0.056 mg/ g fresh weight callus ± 0.0009 and 0.0387mg/ g fresh weight callus ± .0056 for inoculated and control respectively. Same results were reported by Hamid et *al*. (2014), and in control proteins amount was generally higher compared to inoculated calli. Lower proteins levels could be correlated to a decline in callus growth.

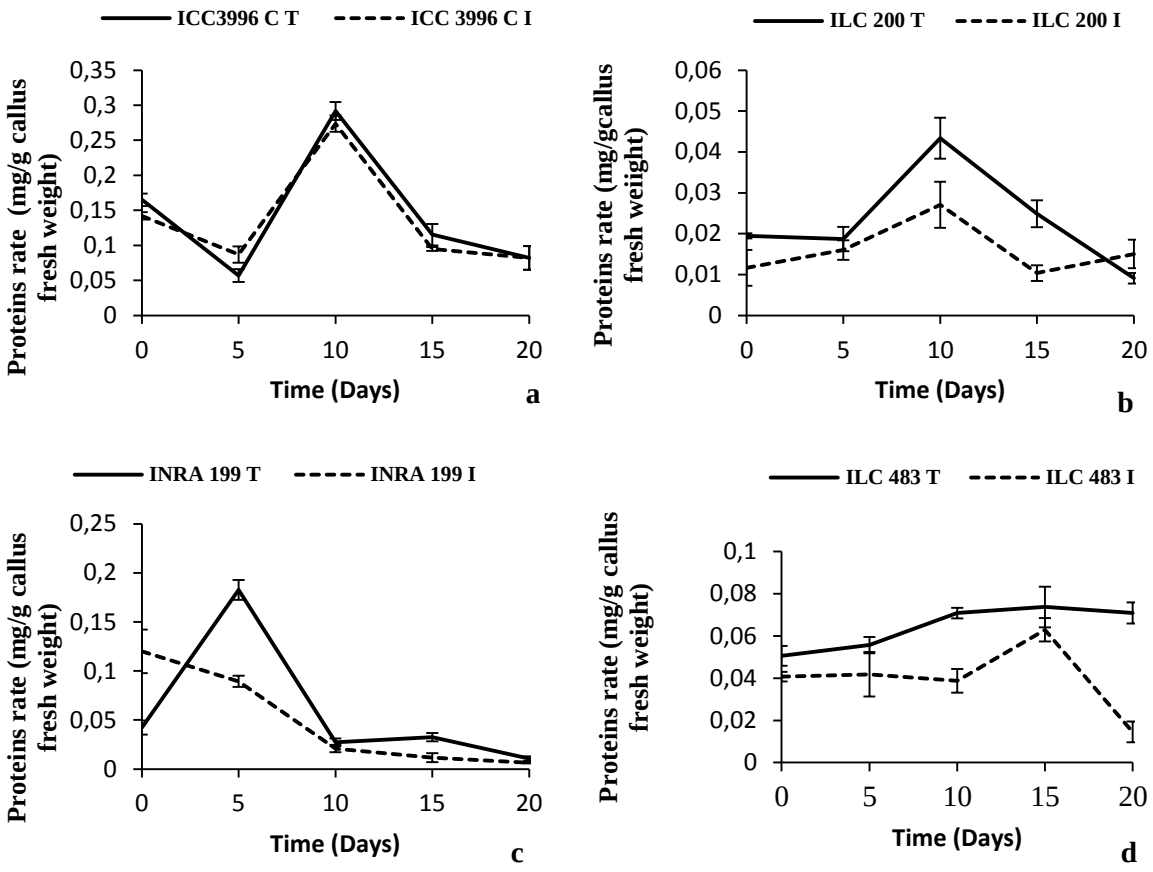

Figure 2 Total proteins rates in control (T) and inoculated (I) chickpea genotypes with *Ascochytarabiei* spores; ICC 3996C (a), ILC 200 (b), INRA 199 (c), ILC 483 (d)

Sugars are not only the main energy source and structural components, but also may control expression of many plant genes and metabolic processes pathogen defense (Platek and Niemczyk, 2000). Sugars may act as priming agents may also be useful in programs to generate stress-resistant cultivars (Morkunasand Ratajczak, 2014). This suggests that increasedsoluble sugar content may be pivotal in the ability ofelicitors to stimulate secondary metabolite biosynthesis Fan *et al.*(2011).

In this experiment, in soluble sugars accumulation as defense response, generally two phases was observed for ILC 483 (Fig.3 b). In the initial days control calli showed generally a decrease in soluble sugars rates.Sugars are probably used intensive respiratory process or for compound connected with interaction response. This biphasic response was reported in many stress reaction (Platek and Niemczyk, 2000). In INRA 199 (Fig. 3 d) characterized as often resistant to *Asochytarabiei*, an increase in soluble sugar amount was observed with significant difference after 10 days in inoculated callus (1.3260.5144 mg/ g fresh weight callus ± 0.0109 ±0.143) with compared to control (0.5144 mg/ g fresh weight callus ± 0.0109)it is generally assumed that these compounds levels are in resistant varieties Surrendranatha Reddy *etal*., 2011).

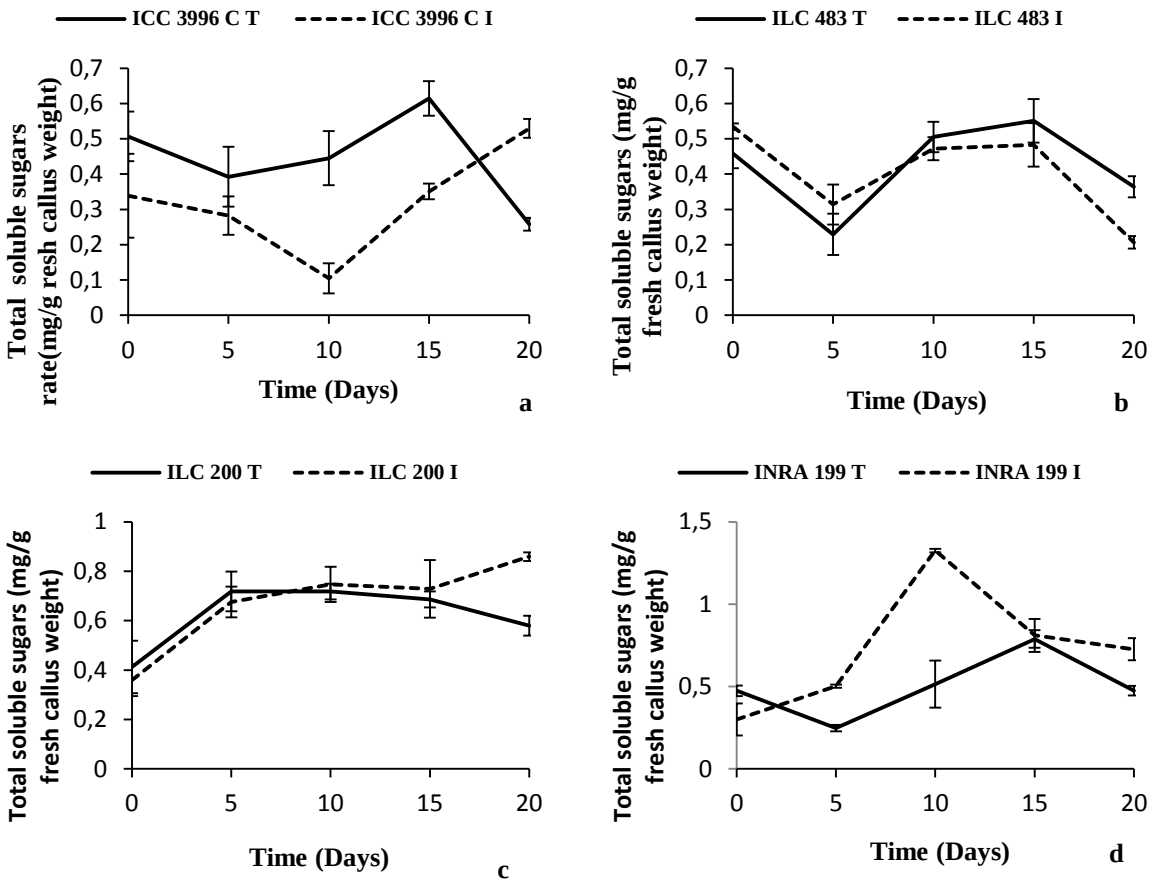

Figure 3 Soluble sugars rates in control (T) and inoculated (I) chickpea genotypes with *Ascochytarabei* spores; ICC 3996C (a), ILC 483 (b), ILC 200 (c), INRA 199 (d).

Phenolic compounds are secondary metabolites related with plant defense (Lattenzio et *al.*, 2006). Their levels are higher in resistant genotypes compared to tolerant and susceptible ones Surrendranatha Reddy et al., 2011).

INRA 199 (Fig.4 a) genotype polyphenols rates decreases gradually with time while for ILC 200 (Fig.4 c), a visible increase in inoculated callus, and for ILC 483 (Fig.5 d) it remained stable until 20th day and then decreased and reached 0.0156 mg/ g fresh weight callus ± 0.0024.The post inflectional increase in phenolic content could be due to their release by pathogen.

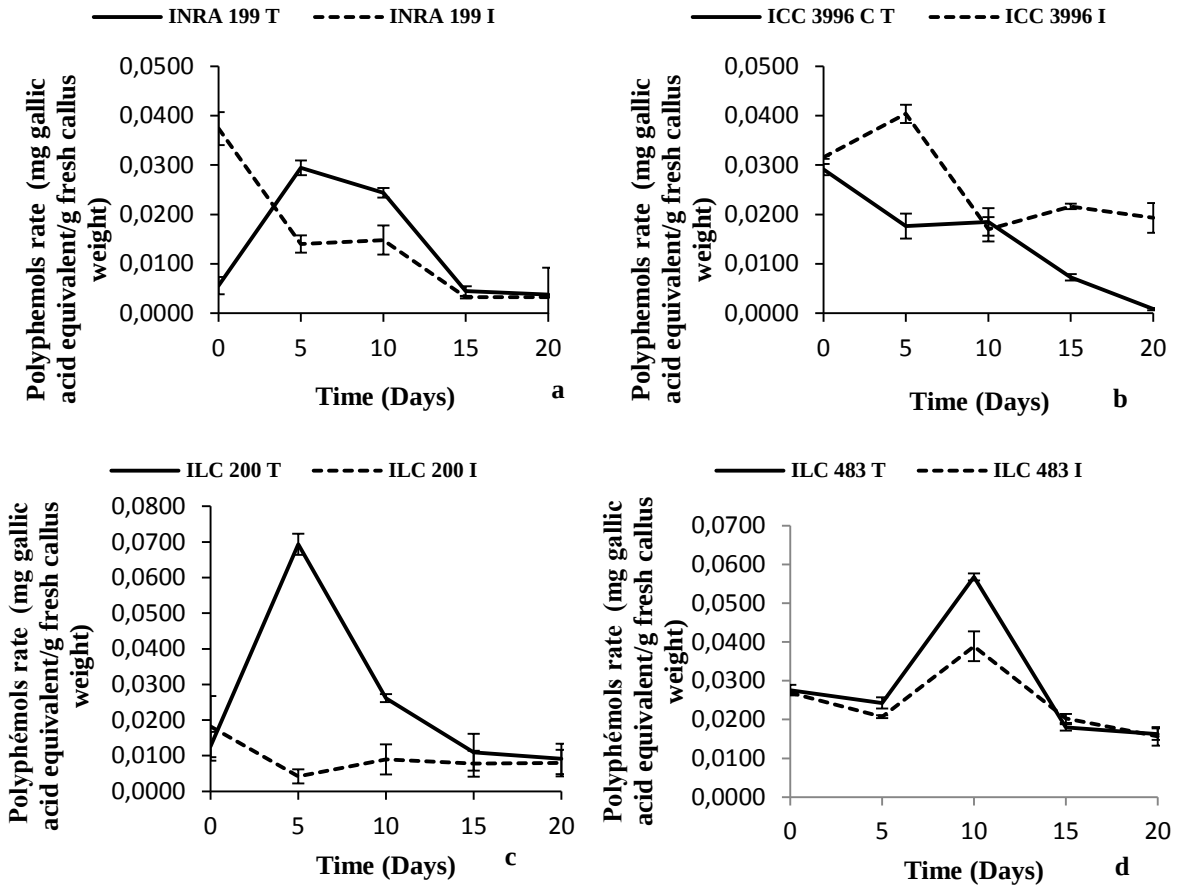

Figure 4 Polyphenols rates in control (T) and inoculated (I) chickpea genotypes with *Ascochytarabei* spores INRA 199 (a), ICC 3996C(b), ILC 200 (c), ILC 483 (d).

Flavonoids

Flavonoidshave recently been implicated as a secondary metabolites involved in plant resistance. (Duressa *et al.*, 2010). In this experiment, INRA 199 (Fig.5 a) showed a distinct significative difference in inoculated calluses then a decrease with interaction time .Same profile was observed with ILC 483 (Fig.5 c). This finding is confirmatory with Mathpal *et al*., (2011) who reported that flavonoids content decreases with age advancement of age in infected plants. For ICC 3996 C (Fig. 5 b), flavonoids rate was different after 10 days of inoculation, then a noticeable decrease was observed even in control (0.022 mg/ g fresh weight callus ± 0.0009) and inoculated callus (0.0189 mg/ g fresh weight callus ± 0.0025).

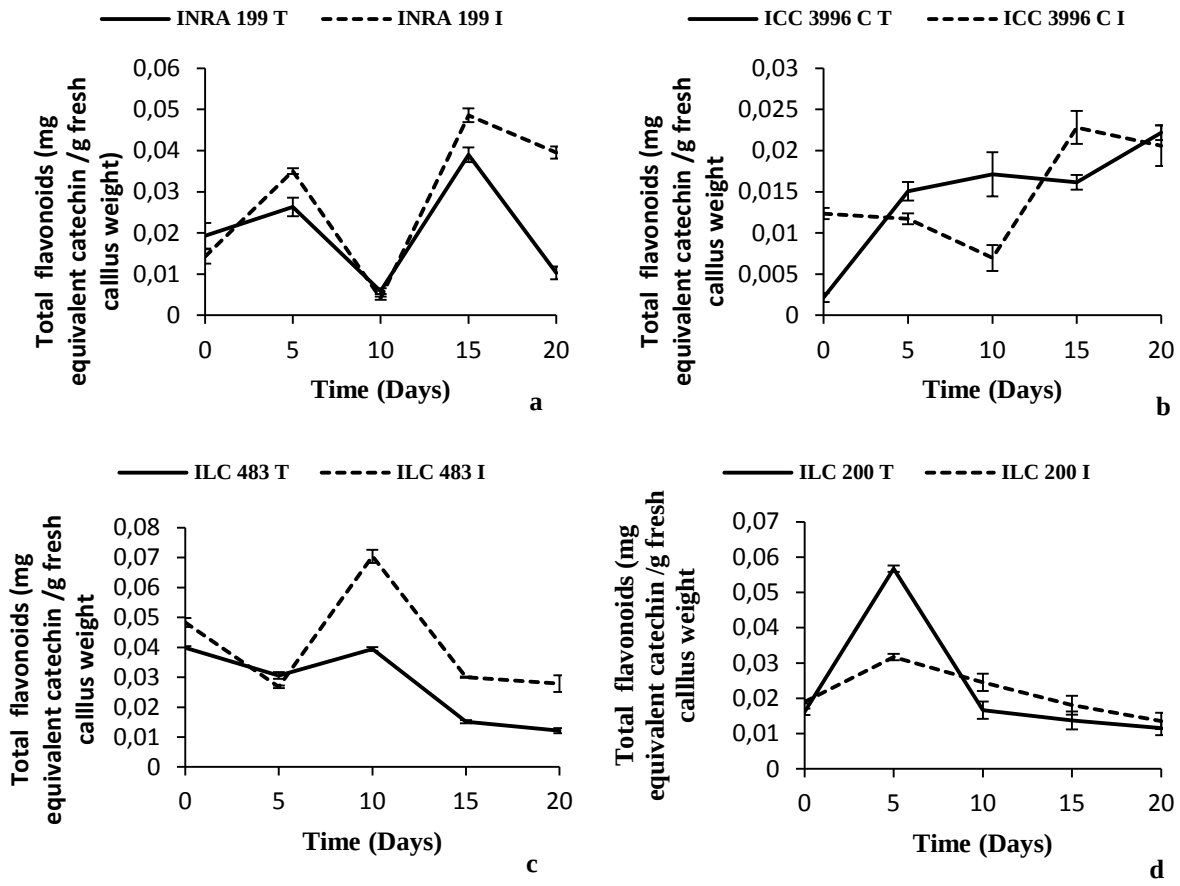

Figure 5 Total flavonoids rates in control (T) and inoculated (I) chickpea genotypes with *Ascochytarabei* spores; INRA 199 (a), ICC 3996C (b), ILC 483 (c), ILC 200 (d).

Using a pathosystem including calli and fungi spore suspension provide a simple method to study host and parasite interaction biochemical profile.

Based on present finding, it may be concluded that the interaction between Chickpea calluses and spores of *Ascochyta rabiei* triggers processes such as proteins, carbohydrates, polyphenols and flavonoids synthesis. These important biochemical compounds are related with host and its parasite relationship.

Their accumulation level seems depending on genotypes used as source of calluses, time of inoculation and parasite nature.

Further investigations especially qualitative analyses may provide more information which can lead to resistant plant selection.

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Original scientific paper
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ORGANOLEPTIC ASSESSMENT OF FIG STRUDEL WHERE SUCROSE WAS SUBSTITUTED WITH HIGH FRUCTOSE SYRUP

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Abstract

The goal of this paper is to perform an organoleptic analysis with fig strudel, which had, during a technological process, substituted sucrose with high fructose syrup in ratio of 20%, 25% and 30% and to carry out the organoleptic analysis of the very product as well as its quality after one and two months long storage. The organoleptic analysis of the samples was carried out by reviewers from Agro Mediterranean faculty by establishing a commission and evaluation sheet with following characteristic listed: colour, taste, smell, structure and the shape, which were then put into table and statistically analysed. The obtained results show that the product with the partial level of sucrose with high fructose syrup of 25% has 88 points and it has an excellent score. However, other products also, both experimental and standard have obtained excellent score. Authors of this paper have carried out organoleptic assessment of products that were storage for more than 30 days, where all the products had scores with minimal variance as with the first analysis. After 60 days of storage, the product with 25% of substituted sucrose had score of 81 points what still have the product excellent quality score, while in other products we noticed the decrease in points what gave them score of very good. Statistical data analysis was carried out by using analysis of variance and based on the obtained results we can conclude that analysed samples are different and that they are products with different characteristics what improves the understanding of the difference in descriptive method of organoleptic assessment of analysed samples.

Key words: *organoleptic analysis, strudel, assessment*

Introduction

This science and research paper is based on an attempt to decrease energetic value of waffle products by substituting sucrose with high fructose syrup that is contained in a very product in a significant percentage. People’s everyday habits are based on consumption on sweet industrial products. Due to the lack of physical activities as well as large number of diabetics, the sweets are being left out from our nutrition, what makes certain categories of population inferior. There is an apparent wish to eat sweets, while our health rules this wish out.

In order to decrease the energetic value of the final product during the process, authors have used sucrose substitute with high fructose syrup in ratio of 20%, 25% and 30% in the strudel filled with fig marmalade (Omanović, 2008). High fructose syrup, in theory, is equivalent to sucrose in terms of sweetness, because it contains glucose and fructose in proportion similar to sucrose with 52% and 42%. The texture of the sweet waffle is more or less strong, more or less sweet and les fragile. It has aromatic and neutral aroma and sweet flavour, (Gavrilovic, 2003). In order to control minimal conditions of the quality standards stipulated by relevant Rulebooks, that stipulates standards for cookies and products similar to cookies. The usage of high fructose syrup in production of cakes has an effect on the flavour, volumes, texture and the colour of the very product. Organoleptic analysis of the tested products will determine the level of acceptability of the product on the market because it represents the decision of the very consumers in terms of the quality of the given product.

Material and methods

Organoleptic assessment of final fig strudel products where we substituted sucrose with high fructose syrup as in the standard product was carried out in 2008 at Agro Mediterranean Faculty of Dzemal Bijedic University of Mostar, City of Mostar, Bosnia and Herzegovina. Organoleptic assessment includes the reaction of human senses to smell, taste, shape and structure what is a result of sending of impulses to central nerve and from it to the very brain where we form and define our impression about the product. Organoleptic analysis was carried out in conditions of controlled moisture, at room temperature with daylight and in serenity. The samples were selected in a separate rove m and then prepared for assessment. In order to carry out an organoleptic assessment, we have formed a commission of (judges) whose senses of smell and taste are normally developed, who are in good health and psychologically stable. The judges had a prior knowledge in regard to assessment material as well as the form of production, different faults and defects as well as the causes of these phenomena. There were in total five judges of different age. In order to neutralise the previous flavours, the judges used clear water. Before the very assessment, the commission adopted the assessment system i.e. the system of grading. The assessment of grading quality was applied for the entire organoleptic assessment by testing and examining of the product. The grade for each characteristic was given in number from one to five. The quality of the product was determined based on the sum of the grades. We measured the organoleptic durability also after 30 and 60 days.

Image 1: products for sensor analysis

Authors used organoleptic analysis to assess the following characteristics of the product:
The shape,
Structure,
Colour,
Smell,
flavour,

Cumulative evaluation sheet was used to enter the results from the evaluation sheets. The judges entered their data into the cumulative evaluation sheet for each sample separately. The quality of the product was determined based on the results that were obtained based on the following grading system:
from 81 to 100 points – excellent
from 61 to 80 points – very good
from 41 to 60 points – good
from 21 to 40 points – satisfactory
from 0,0 to 20 points – dissatisfactory

The data obtained through organoleptic assessment were statistically processed. Authors used the ANOVA analysis (analysis of variance) to test the population of the products, i.e. whether they belong to the same population or not.

Results and discussion

After the sensory analysis of all treated products, the obtained results are shown in the following table:

Table 1 results of the organoleptic assessment of the fig strudel

PRODUCT	***CODE***	***NUMBER OF JUDGES***					***TOTAL SCORE***
		1	***2***	***3***	***4***	***5***	
FIG STRUDEL	***PR-1 25%***	***20***	***16***	***19***	***16***	***17***	***88***
FIG STRUDEL	***PR-230%***	***18***	***17***	***16***	***16***	***18***	***85***
FIG STRUDEL	***PR-320%***	***18***	***18***	***17***	***15***	***18***	***86***
FIG STRUDEL	***PR-4 standard***	***19***	***18***	***17***	***13***	***20***	***87***

Table 2 summary assessment of the product per characteristic

Product	**Shape**	**Structure**	**Smell**	**Flavour**	**In total**
P1	21,00	22,00	23,00	22,00	**88,00**
P2	18,00	21,00	25,00	21,00	**85,00**
P3	20,00	23,00	20,00	23,00	**86,00**
P4	22,00	23,00	21,00	21,00	**87,00**

After summation, product with code PR-1 had 88 points, this implies that product with 25% of sucrose substituted with high fructose syrup received an excellent quality grade. However, other products as well both experimental and standard received excellent quality grade.

Authors have also carried out the organoleptic assessment of the product after 30 days where all products received identical grades as after the first summation. Analogously, the product with code PR 1 had the highest score again and was assessed as a product of excellent quality. Therefore, we can conclude that during the 30 days period there were no significant changes in terms of product quality.

The grading after 60 days gave the following results:

PR-1 total score of 81

PR-2 total score of 78

PR-3 total score of 79

PR-4 total score of 80

After 60 days of conserving the product with 25% sucrose substituted with high fructose syrup PR1 received a score of 81 what gave it a grade of excellent quality, while other products suffered a decrease in the summation and received a very good quality grade. .

The data obtained through organoleptic assessment were statistically processed. Authors used the ANOVA analysis (analysis of variance) to test the population of the products, i.e. whether they belong to the same population or not.

Authors have tested hypothesis H0 (that all products belong to the same population) as opposed to the hypothesis H1 (that products do not belong to the same population).

Table 3: Results of the statistical data processing

ANOVA: Single Factor

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	4	17,6	4,4	0,026667
Row 2	4	17	4,25	0,33
Row 3	4	17,2	4,3	0,12
Row 4	4	17,4	4,35	0,036667

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,05	3	0,016667	0,12987	0,940486	3,490295
Within Groups	1,54	12	0,128333			
Total	1,59	15				

Result implies to reject the H0, therefore we can conclude that we are dealing with product of different characteristics.

Conclusion

Strudel filled in with fig marmalade where we substituted sucrose with high fructose syrup were assessed in three different series according to the given variables, shape, structure, smell and flavour. Organoleptic assessment of the product was carried out immediately after the production, but also after thirty, that is sixty days.

The results have shown that the product with partial substitution of 25% sucrose with high fructose syrup had the biggest number of points – 88, what means that it was graded as the product of excellent quality, while other products both experimental and standard had somewhat less scores.

Authors carried out an organoleptic analysis of experimental and standard products after thirty days in conditions of set storage. The analysis showed that all products kept their excellent quality. Therefore, we can conclude that there were no changes in terms of product quality.

Organoleptic analysis after sixty days have shown that product with 25% of high fructose syrup has maintained its excellent quality while other products were graded as very good. We can conclude that they suffered a change and decrease in quality.

The data obtained through organoleptic assessment were statistically processed and the results of ANOVA analysis show that the products have different characteristics.

The judges decided that the tested products have a good quality and recommend them for the market placement.

Even though, sensory analysis is somewhat subjective, this method is important because it represents a consumers' judgement regarding the quality of certain product.

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ASSOCIATION OF CYNODONO-SORGHETUM HALEPENSAE (LABAN 1974) KOJIĆ 1979 IN THE VINEYARDS OF BOSNIA AND HERZEGOVINA

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Abstract

This paper presents a structure and floristic composition, biological spectrum, gradient analysis, areal range, numerical classification and ordination of widespread association *Cynodono-Sorghetum halepensae* (Laban 1974) Kojić 1979 in wine-growing regions of Bosnia and Herzegovina. It was taken a 32 relevés, and characteristics of the investigated sites date are presented. The total number of 79 plant species make floristic structure of the association. Typical types of associations are: *Cynodon dactylon* (L.) Pers. and *Sorghum halepense* (L.) Pers. In composition of the association apart of characteristic species, *Convolvulus arvensis* L. was present. Biological association spectrum shows domination of terophytes and hemicryptophytes. Gradient analysis shows the greatest dependence on floristic composition of the association in related with soil moisture. Areal spectrum of association are characterized by the dominance of species of wide distribution. Numerical classification of analyzed association can clearly distinguish three groups of sites, which is confirmed by the results of ordination that was performed correspondent analysis.

Key words: *correspondent analysis, vineyards, weed associations.*

Introduction

The study of weed flora and vegetation of the vineyard were less studied in comparison to other agrophytocenoses. However, a number of authors has made a significant contribution to understanding the characteristics of the weed of vineyards. Those research papers in one way was related to the state and prospects of viticulture and general questions about the characteristics of weed in vineyards (Tarailo, 2001), while in the other way they performed floristic and phytocoenological research (Jovanović, 1999; Dujmović-Purgar and Hulina, 2004; Pál, 2004; Lososová et al., 2004; Kovačević, 2008), often related to the monitoring of seasonal dynamics (Crnčević et al., 1992; Lososová et al., 2003) and indicative role between microhabitats (Ubavić et al., 2004). A large number of research were related to the effects of herbicides use, economic aspects and tillage methods (Poldini et al., 1998; Leeson et al.,1999; Baumgartner et al., 2007; Baumgartner et al., 2008; Vrbničanin et al., 2009). A numrous papers were related to synmorphologycal changes in weed communities, as well as changes of taxonomic position, as result of applying of herbicides for many years (Ajder 1992). Our research covers the area of the two wine regions: the northern region of Bosnia and region of Herzegovina, splited due to morphostructural properties clearly differ in terms of climate and soil characteristics. The aim of the present study is the floristic-phytocoenological research of weed flora and vegetation of vineyards, which should provide current status of weeds amount, floristic composition and structure of weed communities, in this completely unexplored area of Bosnia and Herzegovina.

Materials and methods

Floristic-phytocoenological research was carried out according to the principles and methods of the French-Swiss phytosociological school of Braun Blanquet (1965). Determination of plant species is done on the basis of publications: Flora Europaea I-V (Tutin ed., 1964-1980), Flora SR Srbije I-IX (Josifović ed., 1970-1977), Flora Hrvatske (Domac 1994), Ikonographie

der Flora des Südöstlichen Mitteleuropa (Javorka and Csapody, 1979) and Flora Italiana (Fiori and Paoletti, 1921). Taxonomy and nomenclature follows Flora Europaea I-V (Tutin ed., 1964-1980). Sintaxonomic review of vegetation is shown acording to publication Sintaksonomski pregled vegetacije Srbije (Kojić et al., 1998). Life forms of plants were determined according to the publication Flora Srbije (Sarić ed. 1992). Floral elements identified species is represented by Oberdorfer (2001). Multivariate statistics was performed by using a computer package program Biodiversity Pro (McAleece, 1998). Numerical classification of the analyzed sites was performed by method of correspondent analysis (Hill 1974) and UPGMA methods (Sneath and Sokal, 1973).

Results and discussion

Floristic structure of the association are shown in table 1. The floristic composition of the association are presented with 79 species. The sites of the association are developed on flat terrain at an altitude of 53 to 281 meters above the sea level. Characteristic species of the association are *Cynodon dactylon* (L.) Pers. and *Sorghum halepense* (L.) Pers. Species *Cynodon dactylon* (L.) Pers., is not typical only for this association, because it has high abudance in other weed associations (Crnčević, 1988). However, in this association it is present in all sites and with very large number and cover value (Cover 4671,54), so its role in the structure of this association is extremely important. Due its biological and morphological properties *Sorghum halepense* (L.) Pers. have considerable influence on the genesis and structure of the association, but much smaller number than *Cynodon dactylon* (L.) Pers. This leads to the fact that mentioned species has a smaller role in structure of this association. A special feature sites of this association is that all three species of characteristic association set are geophytes. Their spreading is probably conected to low level of agricultural practices and their morphological characteristics (Baumgartner et al. 2007).The composition of the characteristic set, besides typical species, also makes the species *Convolvulus arvensis* L.

Table 1. Floristic structure of the association

Species	Sites																															Pr.	Cover value
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32		
Characteristic species of association																																		
Cynodon dactylon (L.) Pers.	7	8	5	7	9	5	7	8	7	8	8	7	9	7	8	7	7	5	7	8	7	9	8	7	8	7	9	7	5	5	7	7	V	4671,54
Sorghum halepense (L.) Pers.	3	5	7	5	3	3	3	2	2	2	.	.	2	.	5	.	.	.	7	3	5	3	3	3	3	3	2	.	.	.	.	.	IV	610,99
Characteristic species bond *Polygono-Chenopodion* Koch 1926 em Sissing. 1946																																		
Setaria glauca (L.) Beauv.	.	.	.	.	3	3	.	3	2	2	.	.	7	7	.	5	7	2	.	.	.	.	.	.	.	.	5	5	2	5	3		III	634,09
Euphorbia helioscopia L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	3	3	7	3	3	5	5	.	.	.	.	.	.	II	343,78
Lamium purpureum L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	2	5	.	2	5	3	2	.	.	.	5	5	3	.	II	266,58
Digitaria sanguinalis (L.) Scop.	.	.	.	3	2	2	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	5	3		I	102,20
Setaria viridis (L.) Beauv.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	5	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	70,63
Fumaria officinalis L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	I	15,63
Diplotaxis muralis (L.) DC.	.	2	.	.	.	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	0,93
Mentha arvensis L.	.	.	.	.	.	.	2	.	.	.	2	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	I	0,93
Chenopodium polyspermum L.	.	.	.	.	.	.	.	.	.	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	0,62
Allium vineale L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	2	.	.	.	.	.	.	.	.	.	I	0,62
Characteristic species ordo *Chenopodietalia albi* Tüxen, Lohm. et Prsg. 1950																																		
Amaranthus retroflexus L.	2	2	7	7	.	.	.	2	.	2	3	3	.	3	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	II	298,45
Chenopodium album L.	2	2	.	.	5	5	3	.	2	2	.	.	.	.	2	2	7	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	II	244,37
Geranium molle L.	.	.	.	.	.	.	.	2	2	.	.	.	.	.	.	5	5	5	3	2	.	5	.	.	.	.	.	.	.	.	.	.	II	235,32
Senecio vulgaris L.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	2	5	.	3	3	3	3	.	3	.	.	.	.	.	.	.	.	.	II	133,46

Portulaca oleracea L.	2 2 3 3 2 2 3 . . . . . 2	II	48,44
Hibiscus trionum L.	. . . . . 2 . 2 2 . . 2 8 . . 2	I	194,86
Capsella bursa-pastoris (L.) Med.	. . . . . 2 2 2 5 3	I	71,25
Echinochloa crus-galli (L.) Beauv.	2 . . 3 3 . . . . . 3 2 2 .	I	47,82
Polygonum lapathifolium L.	2 . . . . . 2 . . . . . 3 . .	I	16,25
Solanum nigrum L.	. 2 . 3	I	15,94
Galinsoga parviflora Cav.	. . . . . 3 . .	I	15,63
Sonchus oleraceus L.	. . . . . 2 . . . 2 . 2	I	0,93
Verbena officinalis L.	2	I	0,31
Characteristic species class *Stellarietea mediae* Tüxen, Lohm. et Prsg. 1950			
Stellaria media (L.) Vill.	. . . . . 8 7 7 7 5 3 5 . . 7 . 8 9 8 .	II	1447,1
Veronica persica Poir.	. . . . . 5 5 5 5 7 5	I	390,64
Anthemis arvensis L.	. . . . . 3 . . . . . 2 . . 3 . . . . 2 .	I	31,88
Viola arvensis Murr.	. . . . . 3	I	15,63
Kickxia spuria (L.) Dum.	. . . . . 2 2 2 . . . 2	I	1,24
Conyza canadensis (L.) Cronq.	. . . . . 2 . . . . 2 . . 2	I	0,93
Bilderdykia convolvulus (L.) Dumort.	2	I	0,31
Others			
Convolvulus arvensis L.	7 7 3 7 5 . 5 2 3 7 5 7 3 3 5 5 2 . . . . 2 7 2 5 5 . . . 3 3 3	IV	1196,62
Crepis sancta (L.) Babcock	. . . . . 5 5 7 3 5 8 8 2 3	II	699,45
Trifolium repens L.	. . . . . 7 3 3 3 . . 3 7 7 . 5 5	II	523,47
Taraxacum officinale Weber	. . . . . 5 . 3 . 3 3 . . . 3 7 7 . 2 3	II	367,53
Rumex crispus L.	. . 3 . . . 2 . . . . 3 . . 2 . . . 2 . . . . . 2 . 5 5 . . 2	II	142,19
Polygonum aviculare L.	2 7 . . 5 9 5 . 2	I	500,63
Medicago lupulina L.	. . . . . 5 . . . 3 5 7 5	I	296,89
Vicia sativa L.	. . . . 7 3 . . . . . 3 . 7 3	I	281,27
Berteroa mutabilis (Vent.) DC.	. . . . . 7 7 . . 3 3	I	265,64
Picris echioides L.	. . . . . 5 2 7	I	172,19
Rorippa sylvestris (L.) Bess.	. . . . . 7 . . .	I	117,19
Heliotropium europaeum L.	. . . . . 5 . . 5	I	109,38
Cirsium arvense (L.) Scop.	. . . . . 2 3 . . . 5 . . 2 . . . . 2	I	71,25
Chondrilla juncea L.	. . 5 . . . . . 2	I	55,00
Potentilla reptans L.	. . . . . 2 . . . 5	I	55,00
Trifolium pratense L.	. . . . . 5 . . 2	I	55,00
Leontodon autumnalis L.	. . . . . 5 . . 2	I	55,00
Glechoma hederacea L.	. . . . . 5	I	54,69
Agropyron repens (L.) Beauv.	 5	I	54,69
Plantago lanceolata L.	. . . . . 3 . . . . . 2 . 3 2	I	31,88
Erigeron annuus (L.) Pers.	. . . . . 3 . 2 . 2 2	I	16,56
Muscari racemosum (L.) Mill.	. . . . . 2 . . 3 2	I	16,25
Achillea millefolium L.	. . . . . 2 . . . . . 2 . . 3	I	16,25
*Tribulus terrestris*L.	. . . . . 3 . . . . . 2	I	15,94
Galium mollugo L.	. . . . . 3 2	I	15,94
Lepidium draba L.	. . . . . 2 3	I	15,94
Amaranthus albus L.	. . . . . 3	I	15,63
Avena barbata Pott. ex Link.	. . . . . 3	I	15,63
Bellis perennis L.	. . . . . 3 . . .	I	15,63
Prunella vulgaris L.	 3	I	15,63
Xanthium italicum Moretti	. . . . . 2 . 2 2 . 2 . . . 2 . 2	I	1,86

Linaria vulgaris Mill.	2 2	I	0,62
Malva sylvestris L.	. 2 . . . . 2	I	0,62
Satureja montana L.	. . . . 2 2	I	0,62
Plantago major L.	. . . . 2 2	I	0,62
Rumex obtusifolius L.	. . . 2 . . . . 2 2 . . . 2 . . 2 . .	I	1,55
Lactuca saligna L.	. . . . 2 . 2 . . . . . 2 2 2	I	1,55
Phleum pratense L.	2	I	0,31
Clematis flammula L.	. . . . . . 2	I	0,31
Raphanus raphanistrum L.	. . . . 2	I	0,31
Datura stramonium L.	. . . . 2	I	0,31
Foeniculum vulgare Mill.	. . . . 2	I	0,31
Rubus ulmifolius Schott.	. . . . 2	I	0,31
Ornitogalum umbellatum L.	. . . . 2	I	0,31
Reseda lutea L.	. . . . 2	I	0,31
Cichorium intybus L.	. . . . 2 . . .	I	0,31
Ambrosia aretemisiifolia L.	 2	I	0,31

The biological spectrum of association shows domination of therophytes and hemicryptophytes, that consitute almost 90% of all species (Table 2). Of the total number of therophytes (45), the highest cover values have following species: *Amaranthus retroflexus* L., *Chenopodium album* L., *Euphorbia helioscopia* L., *Lamium purpureum* L. and *Hibiscus trionum* L.

Table 2. Biological spectrum of association

Life forms	The number of species	%
Therophyte	45	56,96
Hemicryptophyte	24	30,38
Geophyte	7	8,86
Scandenophyte	2	2,53
Phanaerophyte	1	1,27

The living area spectrum of association are characterized by the presence of nine groups of floral elements (Table 3) with the dominance of species of wide distribution. Most of the weeds in both studied areas belogs to the cosmopolitan element, followed by euro-asian, mediterranean and adventive.

Table 3. Floristic elements of association

Floristic elements	The number of species	%
Cosmopolitan	27	34,18
Euro-Asian	12	15,19
Mediterranean	12	15,19
Adventive	11	13,92
Boreal	6	7,59
Sub-Mediterranean	6	7,59
Sub-Atlantic	3	3,80
Continental	1	1,27
Moderate Continental	1	1,27

Correspondence analyses (Figure 1) performed on the basis of weed species of the higest abundance and coverage has showed the floristic diferences in vegetation of the studied areas. As floristically similar sites it is possible to separate the first group of sites (marked by numbers from 1 to 17), the second group of sites (marked by numbers from 18 to 27) and the third group sites (marked by numbers from 28 to 32), as it is presented in the dendrogram (Figure 2).

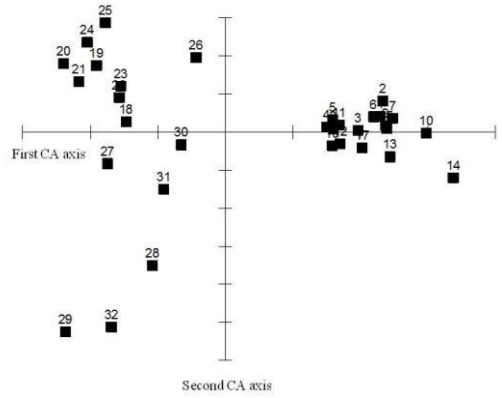

Figure 1. Ordination of sites association

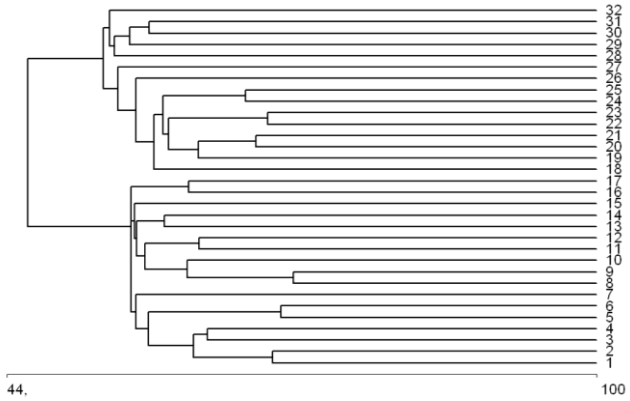

Figure 2. UPGMA classification sites of association

Sites in the 2 areas were grouped together with distinctive feature of the 3 areas. The first group of sites is typical for the summer and autumn aspect of the vineyards of Herzegovina, and it is reflected by the presence of a large number of species characteristic for higher syntaxonomical unit. Second group of sites is characteristic for the spring aspect in the vineyards of Herzegovina. The third group of sites is typical for the summer and autumn aspect of the vineyard of northern Bosnia.

Conclusion

In Bosnia and Herzegovina the association *Cynodono-Sorghetum halepensae* (Laban 1974) Kojic 1979 is typically developed in the vineyards of Herzegovina, primarily among the microhabitats of row in which chemical weed control dominante. In the vineyards of northern Bosnia sites this associations are developed over the entire surface. They partly have the character of meadow type, caused by low-intensity of agricultural practices.

The sites of the association in the vineyards region of Herzegovina are usually developed in the microhabitats of row, because the intet-row is intensively farmed. The analysis range of the spectrum has showen importance of cosmopolitan and Eurasian species in this association. In floristic composition of association influence of mediterranean area is obviosly.

From a cosmopolitan group of floral elements the greatest cover value have: *Cynodon dactylon* (L.) Pers., *Stellaria media* (L.) Vill. and *Convolvulus arvensis* L.

Numerical classification of the analyzed sites of association *Cynodono-Sorghetum halepensae* (Laban 1974) Kojić 1979 clearly distinguish three groups of sites, which is confirmed by the Correspondence analysis.

Great importance of the association *Cynodono-Sorghetum halepensae* (Laban 1974) Kojić 1979 is related to the great number of geophyte species (*Cynodon dactylon* (L.) Pers, *Sorghum halepense* (L.) Pers. and *Convolvulus arvensis* L.), their biological characteristics as they presence in the whole growing seasons, which is a major problem in taking measures to weed control especially among microhabitats.

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SCREENING FOR ANTIXENOSIS RESISTANCE OF WINTER WHEAT GENOTYPES TO CEREAL LEAF BEETLES (OULEMA SPP.)

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Abstract

The crop damage caused by cereal leaf beetles of the genus *Oulema* Gozis 1886 (Coleoptera: Chrysomelidae) has been increased in recent decades in different regions of Europe and North America. The purpose of these studies was to evaluate the antixenosis resistance to cereal leaf beetles in modern and old cultivars, local forms and breeding lines of winter wheat (*Triticum aestivum* L.) created in Bulgaria. The investigations were conducted under field conditions by monitoring and reporting on natural infestation of existing local populations of cereal leaf beetles on wheat plants. The extent of differences between 54 genotypes was established to the infestation of cereal leaf beetles and their host suitability. The lowest density of cereal leaf beetles was observed in cultivar Enola (1.2 ind./m^2), breeding lines №301 (1.3 ind./m^2) and Sofia № 312 (2.8 ind./m^2), while the highest was found in cultivar Nedan (22 ind./m^2).
Results of our investigations can contribute to the identification of genotypes as host plants of the cereal leaf beetles and their usage in the bridging process.

Keywords: antixenosis, winter wheat, *Triticum aestivum* L., *Oulema* spp.

Introduction

Common winter wheat *(Triticum aestivum* L.*)* is the staple cereal crop in many countries of the world including all European countries. The wheat production is accompanied by a number of abiotic and biotic stress factors. The technologies of ecological and organic farming and the plant resistance to biotic stress factors are becoming more current and important.

In recent decades in several European and North American regions, the crop and economic damage caused by cereal leaf beetle is increasing. The cereal leaf beetles *Oulema melanopus* (Linnaeus 1758) and *Oulema gallaeciana* (Heyden 1879) (Coleoptera: Chrysomelidae) are oligophagous insects, whose host plants belong to the family Gramineae, but preferring wheat, barley and oats. In these agroecosystems, they are pests with economic importance. The phase of population gradation of *O. melanopus* and important economic damage were reported in North America (Olfert et al. 2004, 2006, Philips et al. 2011, Onychko 2012). The crop damage from cereal leaf beetles in certain areas of Europe could also lead to yield losses (Huusela-Veistola 2010, Tanasković et al. 2012, Onychko 2012). In a mass attack caused by *Oulema* spp., the assimilation processes of infested plant can be reduced up to 80% (Campbell et al. 1989) and yield losses reach 1t/ha, or 0.5-4% in winter wheat and 3-8% in barley (Ulrich et al. 2004).The current control methods of this pests are mainly chemicals. The natural enemies (predators and parasitoids) have not a determining role in lowering the density or maintain them below the economic threshold (Laznik 2010).

The modern tendency in the wheat breeding is not only aiming to increase the productivity of cultivars and to improve grain quality, but also to create plant cultivars, which are resistant to pests.

As the result, the effectiveness of plant breeding increases significantly, because the additional costs for the application of chemicals (insecticides) are not required and thus

reduces the danger of environmental contamination. In this aspect, the growing of resistant varieties is a desirable non-chemical control method.
The purpose of these study was to evaluate the antixenosis resistance to cereal leaf beetles in modern and old cultivars, local forms and breeding lines of winter wheat (*T. aestivum*) created in Bulgaria. The establishment of the resistance of wheat varieties and lines to biotic stress factors and the causes of specific reaction of genotypes, suggest studies on the suitability of various wheat genotypes as host plants of the cereal leaf beetles.

Materials and methods

Field trials and experimental genotypes

Studies were conducted under field conditions in the experimental field in Lozen, Sofia ($42^{o}60`16``$ N, $23^{o}48`27``$ E). The field observations were carried out in period 2010-2013 by assessment of the natural infestation by existing local populations of cereal leaf beetles on various wheat varieties and breeding lines.
As experimental materials 54 genotypes of *T. aestivum* were used, including 31 old cultivars and 8 local forms of wheat, selected and grown in the first half of the 20^{th} century and 15 modern cultivars created since 1960 in the main breeding centres of Bulgaria. Each genotype was sown in triplicate plots. The plots were consisted of two rows spaced 20 cm apart with 25 seeds/row spaced 20 cm within rows. Genotypes were randomized within three repetitions.

Estimation methods

After the overwintering period of adults, the monitoring and assessment were carried out every 5 days. The species *Oulema melanopus* (Linnaeus 1758) and *Oulema gallaeciana* (Heyden 1879) (Coleoptera: Chrysomelidae) were established in a mixed population. The dynamics of number of settled individuals (different development stages of cereal leaf beetles: larvae, pupae and adult) was followed by visual observation of the tested cultivars and lines. The degree of attack (infestation) was estimated by visual counting of the number of each development stages and presented as total average number of individuals per m^2.
The degree of damage was determined during the phase of flowering (BBCH 61-69) after the development of the larvae and was defined as a percentage of leaf area damaged by cereal leaf beetles over the entire surface of the leaf flag. The percentage of infested plants was evaluated on a scale ranging from 1 to 5 (Rouag et al. 2012).

Statistical analysis

The results were analysed using the software package “Statistica 7” (StatSoft 2005). A one-way analysis of variance and Tukey's Test were used to determine the influence of various factors on the investigated characteristic.

Results and discussion

The results of the number of development stages of cereal leaf beetles in the tested genotypes (cultivars, lines and local forms) are presented in Fig. 1 as the total average number of individuals/m^2 for the period 2010-2013. All plants in entire plots were sampled during the BBCH growth stages 31÷89 on assessment date. It should be noted that the species of larvae could not be visually identified. Furthermore, the pupae of *O. melanopus* were not reported, because this species pupate in the soil. The first adults of both species were reported in early May in cultivars Pobeda, Maritsa and Knezha. Adults were observed after the second half of May, in Enola and Rusalka cultivars. The tested genotypes during this period were in growth stage BBCH 47÷59. Their colonizing ability on the plants was so that the highest number was observed in BBCH 69÷71 at 80% of genotypes. The lowest number of individuals was counted in Enola (1.2 ind./m^2), №301 (1.3 ind./m^2), Sofia № 312 (2.8 ind./m^2), and the

highest in Nedan (22 ind./m^2). The adults of the new generation were found to BBCH 89 (fully ripe).

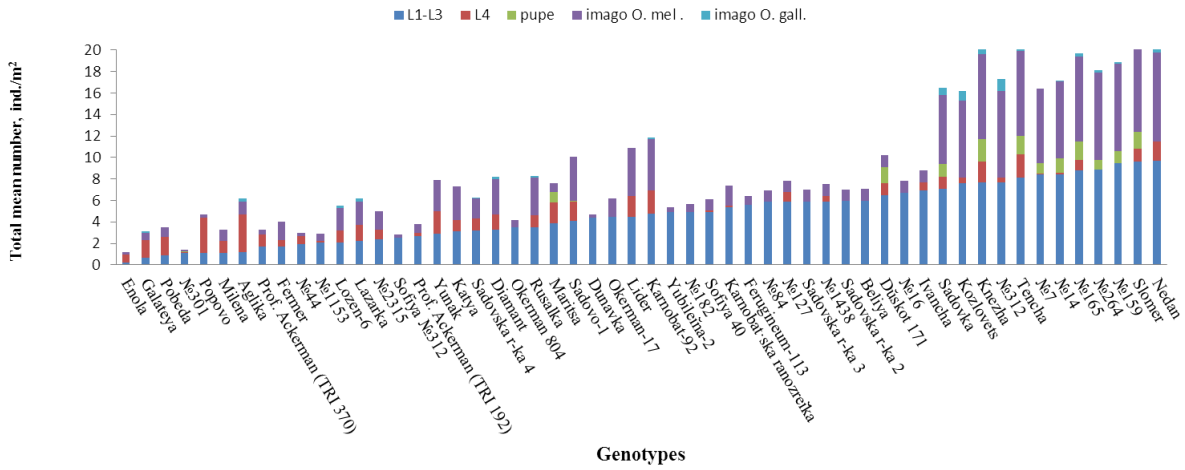

Figure 1. Degree of attack by cereal leaf beetles (*Oulema* spp.) in growth stage BBCH 31÷89 in 54 genotypes *T. aestivum*

The highest infestation level was recorded also in Sadovka №14, №312, №264, №159, №165, Knezha, Slomer, Tencha. Sortovete Diamant, Rusalka, Ivancha, Sadovo-1, Dŭskot 11, Lider, Karnobat-92, Kozlovets at which were reported by 8.2 ind./m^2 to 16.2 ind./m^2 and they occupy an intermediate position as suitable hosts.

The Analysis of variance of the data showed that the effect of genotypes on degree of attack is not statistically significant (Tab. 1). The absence of a significant difference allows us to conclude that the studied genotypes do not differ in their suitability as host plants for the cereal leaf beetles.

Table 1. Analysis of variance relationship between degree of attack by *Oulema* spp. and 54 genotypes *T. aestivum*

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Genotype	10771.6	53	203.2	0.952	0.572ns
Residual	23066.7	108	213.6		
Total	33838.3	161			

Unlike plant pathogenic microorganisms, the insect pests can move actively and choose purposefully their host plants. The mechanisms of resistance to cereal leaf beetles in wheat have been extensively studied (Papp et al. 1992, Delaney et al. 2013, Kher et al. 2014). A lot of investigations found that the resistance to the cereal leaf beetles is conditioned by the presence of trichomes with a specified length and density on the epidermis of the leaves (Wellso 1973, Hoxie et al. 1975, Webster et al. 1972, Haynes and Gage 1981). Wheat varieties with shorter and fewer trichomes are more preferred as oviposition hosts for *O. melanopus* (Hoxie et al. 1975), and these with high tricome densities reduced egg laying rate by 90% or more (Gallun et al. 1973). Those plants make the insect difficult to achieve in the leaf epidermis; the time required for feeding and development of larvae increases; the presence of trichomes limited the movement of larvae on plant surfaces and they can fall from the plants. Because of the presence of leaf pubescence, the wheat can be classified as a cereal

plant with a higher degree of resistance to *Oulema* spp. compared to oats and barley (Konyspaevna 2012).
Production of volatile compounds by some host plant species can have antixenosis effect on pest insects. In most cereals, a secondary volatile substance is DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) (Jonczyk et al. 2008, Meihls et al. 2012). Additionally, in a form of antixenosis, the larvae migrate away from high-DIMBOA maize varieties toward those with low DIMBOA content (Robinson et al. 1978). In corn, DIMBOA also has an antibiotic effect and negatively affects longevity of overwintered *O. melanopus* adults (Wellso 1978).

The degree of damage on the leaves of 54 genotypes showed variation in average values in wheat genotypes from 3 to 43% and the total average for all genotypes was 21.1% (Fig. 2).

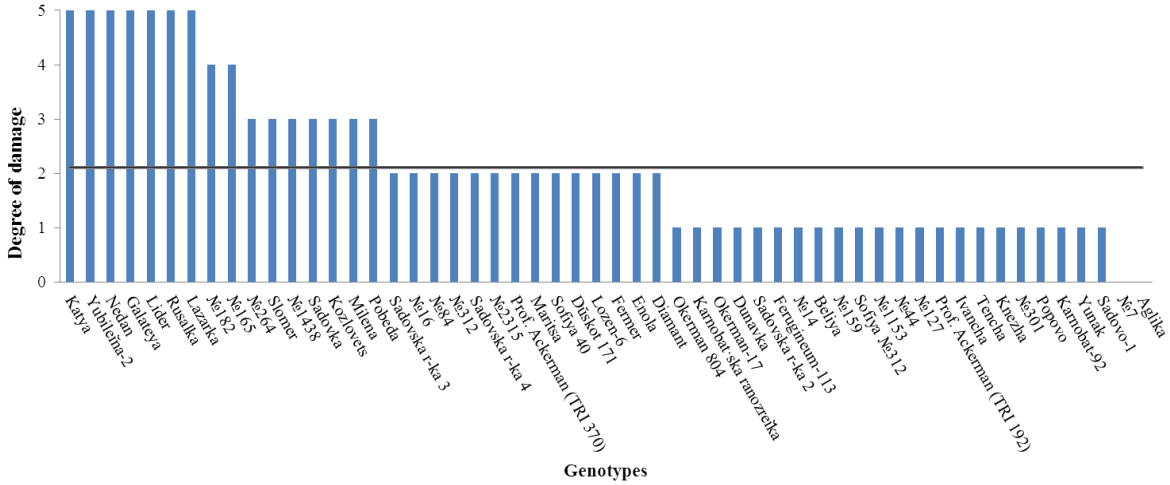

Figure 2. Degree of damage by *Oulema* spp. in 54 genotypes *T. aestivum* ; the line marks the average value

Very low damages were reported on one of the oldest Bulgarian cultivar №7, which was established in 1911. The old cultivars Okerman (Prof. Ackerman TRI 192) and Aglika (Breeding centre Dobrudzha Agricultural Institute, General Toshevo) were with a low degree of damage (9 % resp.0.2 %). The highest damage (43 %) was found on the old cultivar №301, and also on the modern cultivars Katya and Rusalka (37%).
In our studies, the observed differences between the genotypes could be explained by a non-preference reaction (antixenosis) of cereal leaf beetles to resistant plants. In this aspect, further is research needed to done in term to identify which are the factors determining the different degrees of attack and damage in inspected genotypes.
The breeding and cultivation of wheat varieties with genetically determined resistance is an alternative approach to control the insect pests and is particularly important for organic wheat production (Serpolay et al. 2011). Local forms and old varieties of wheat have developed adaptation mechanisms to specific environmental conditions of the area and resistance to plant pests. It is considered that they are a source of genes for improvement of modern varieties as regards resistance to biotic and abiotic stresses (Newton et al. 2010).
Results of our research showed that after comparing the group local forms and old cultivars with the group of modern cultivars no significant differences in the degree of damage by cereal leaf beetles was found (Fig. 3). This shows that modern selection of wheat has not created prerequisites for increasing of the attack and damage on the new cultivars compared with the local forms and old cultivars.

The ecological approach to control of insect pests required the conclusion that the genotypes with moderate resistance, coupled with good wheat properties and quality, are more promising and suitable for application implementation in practice.

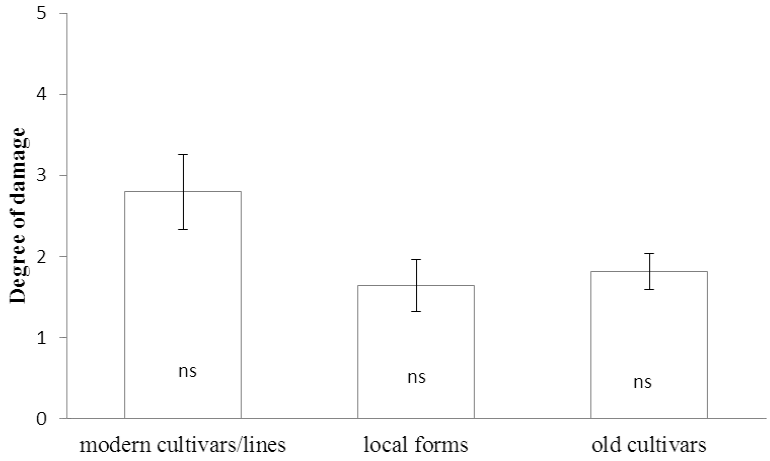

Figure 3. Comparing the genotypes of wheat to degree of damage caused by cereal leaf beetles (*Oulema* spp.); Mean-±SE, Tukey test, $P < 0.05$

Conclusions

The genotypic characteristics of the 54 cultivars, breeding lines and local forms of winter wheat have not shown statistically significant antixenosis effect on settlement preference.

The examined plants were analyzed for assessing the degree of attack and of damage caused by cereal leaf beetles. The lowest number of individuals was observed in cultivar Enola (1.2 ind./m^2), breeding lines №301 (1.3 ind./m^2) and Sofia № 312 (2.8 ind./m^2), and the highest wad found in cultivar Nedan 22 ind./m^2. The smallest damage was reported in Aglika and №7, and the largest in Katya and Yubileĭna-2.

The local forms and old cultivars of wheat do not differ significantly from modern cultivars about the degree of damage caused by cereal leaf beetles.

Results of this examination can contribute to the identification of genotypes as suitable hosts of cereal leaf beetles and their usage in the bridging programs.

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INFLUENCE OF DIFFERENT HERBICIDES ON THE GROWTH AND YIELD OF WHEAT

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Abstract

Within the period 2011-2014, field experiments were conducted at the experimental base of the Agricultural University, Plovdiv. We studied the effectiveness and selectivity of 9 different herbicides applied to the leaves in order to control the weeds and their influence on the growth and yield of wheat of the Diamant variety.

It was established that during the period of the experiment, the height of the wheat plants varied from 75,1 to 79,9 cm as the highest values were those of the herbicides Pasifica BG and Axial 1 plus EK and the lowest values were registered after applying Laren 20 SL.

The most efficient herbicides in the fight against the weeds in the winter wheat are Pacifica WG in a dose of 350 g/ha (79,2%), Pallas 75 WG in a dose of 200 g/ha (76,2%) and Axial 1 plus 050 EK (73,8%). They control 100% of the weeds Avena fatua L. and Alopecurus myosuroides Huds. The weakest is the effect of the herbicide Axial 050 EK in a dose of 600 cm^3/ha (16,7%), because it controls only the monocotyledonous weeds and their density among the crops is low.

The best results regarding the yield were obtained after applying the herbicides Pasifica BG (5214 kg/ha), Sekator OD - 5200 kg/ha and Axial 1 plus 50 EK - 5186 kg/ha. The lowest yield was obtained from the variants treated with Axial 050 EK - 4067 kg/ha and Laren 20 SL - 4665 kg/ha. The results correspond to the data about the effectiveness of the tested herbicidal preparations.

Key words: *wheat, herbicides, growth, yield*

Introduction

Weeds are the main competitors of the winter wheat regarding the nutrients, the water and the light. Their negative influence increases when applying inefficient methods to fight them, which include mainly the use of selective herbicidal preparations (Tonev T. et all, 2007; Qazi, M. et all, 2002; Cheema, M.S. and M. Akhtar, 2005).

The weeds species are changed under the influence of the ecological conditions, agricultural activities and crop rotations (Mitkov A., Tonev T., Tityanov M., 2010). Application of the leaf herbicides in winter wheat is important factor for the weed control (Besolds B., 2002; Dimitrova M. and T. Georgieva, 2005; Haker E.,1999; Khormann P.,2002; Mitkov A.,2012; Weber R., Kieloch R., 2013).

The high level of weed presence directly affects the growth, the development and the yield obtained from grain crops (Dimitrova M. and T. Georgieva, 2005; Haker E.,1999; Khormann P.,2002; Mitkov A.,2012; Weber R., Kieloch R., 2013). This necessitates exercising control over weeds by using various selective herbicides for the purpose of restricting their negative influence on the production results.

For this reason it is necessary to study new possibilities for chemical weed control and to establish the influence of different herbicides on the growth and yield of wheat.

Materials and methods

Conducting the experiment

Within the period 2011-2014 in the experimental field of the Agricultural University, Plovdiv, we made field experiments using new herbicides applied to the leaves during the vegetation period of the wheat, variety *Diamand*. The sowing during the three years of the experiment was performed on October 20-30. The experiments were made using the block method over an area of 21 m^2 in four repetitions (table 1).

Table 1. Herbicide treatments

Herbicide treatment		**Active substance and quantity (*g kg^{-1}; ^{x} g $litre^{-1}$)**					**Dose per hectare**	
							g	**cm^3**
1	Derby super	*	*florasulam*	150.2	aminopiralid	300.5	33	
2	Arat	*	*dicamba*	500	tritosulfuron	250	100	
3	Laren 20 SG	*	*metsulfuron-methyl*			200	30	
4	Secator OD	x	*amidosulfuron*	106	*iodosulfuron*	25		100
5	Lintur 70 WG	*	*triasulfuron*	41	*dicamba*	659	150	
6	Axial 1 plus 050 EK	x	*pinoxaden*	45	*florasulam*	5		1000
7	Axial 050 EK	x	*pinoxaden*			50		600
8	Pallas 75 WG	*	*piroxulam*			75	200	
9	Pacifica WG	*	*mezosulfuron*	30	*iodosulfuron*	10	350	
10	Control		untreated				0	0

The agrotechnical activities were conducted in accordance with the commonly used technology for wheat (processing of the soil, fertilization, sowing, rolling). The herbicides were applied using a knapsack sprayer and a solution of 300-400 l/ha. The efficiency of the herbicides was registered in sample areas (2 for each repetition), i.e. 8 per variants on the 30-th days after spraying.

Method ANOVA was used to determine the significance of the differences between the tested variants (Fowler, Jand L. Cohen, 1992).

Results and discussion

During the period of the experiment, a total number of 16 types of weeds were established in the experimental fields sown with wheat, as the following annual deciduous weeds were predominant: *Veronica hederifolia L., Stellaria media L, Capsella bursa pastoris L., Chamomilla recutita l, Galium aparine l, Agrostemma githago l, Fumaria officinalis l, Papaver rhoeas L., Lamium amplexicaule L.*, and the density of the following annual cereal plants was lower – *Avena fatua L., Lolium temulentum L.* and *Alopecurus myosuroides Huds.* The highest was the density of *Veronica hederifolia L., Stellaria media L, Chamomilla recutita L. and Lamium amplexicaule L.* The variety of the perennial plants was smaller. The following wheat weeds were found: *Johnson grass - Sorghum halepense L.* and the deciduous weeds *Cardaria draba L., Convolvulus arvensis L.* and *Cirsium arvense L.* (table 2).

The applied herbicides control the weeds to a different extent depending on the scope of their activity. The preparations Pacifica WG, Pallas 75 WG and Axial 1 plus 050 EK, which fight not only deciduous but also wheat weeds, control 100% of *Avena fatua L.* and *Alopecurus myosuroides Huds.*. The herbicides used to fight deciduous weeds such as Derbi super, Lintur

70 WG, Arat, Laren 20 CG and Sekator OD, have a good control over the annual deciduous weeds as their density is 2-3 times lower compared with the density of the control sample. Regarding the herbicide Axial 050 EK, which is used to fight only wheat weeds, the density of the deciduous weeds is high and close to the density of the control sample. The density of the weeds in the zero control sample reaches 43.2 plants/m^2.

The growth of perennial weeds is very uneven, which is typical of these types. The perennial wheat weeds are represented by the *Sorghum halepense L.* only. This weed is not affected by the used herbicides and its density ranges from 4.0 to 11.7 plants/m^2.

The used herbicides have a good effect on perennial deciduous weeds, reducing their density to 0.3 – 1.0 plants/m^2 compared with the density of 6.0 plants/m^2 of the zero control sample. The only exception is variant 7, where the applied herbicide is used to fight wheat weeds. The heavy rainfall during the period following the application of herbicides (84.6 l/m^2 in April 2013 and 123.0 l//m^2 in 2014) provided favourable conditions for the repeated growth of weeds.

During the three years of the experiment, the herbicides that proved to be the most efficient in the fight against weeds, compared with the control sample, are Pacifica WG in a dose of 350 g/ha (79.2%), and Pallas 75 WG in a dose of 200 g/ha (76.2%), followed by Axial 1 plus 050 EK (73.8%). The weakest was the effect of the herbicide Axial 050 EK in a dose of 600 cm^3/ha (16.7%). This is due to the fact that this herbicide controls only annual wheat weeds and their density within the experimental field is relatively low.

In table 3 below, we have presented the results showing the effect of the tested herbicides on the formation and the manifestation of the main features determining the productivity of wheat. In the scheme illustrating the experiment, we have compared the variants with the zero control sample – variant 10. We have conducted a biometric analysis in order to study the effect of the herbicides on the features: height of the plant, number of grains and weight of the grains on the class.

The provedness of the differences in the features has been assessed using the method of Student (Shanin, 1977).

We can see that the lowest values of the studied features were obtained in the untreated variant (№ 10) and the highest values were registered in variant № 9 (Pacifica WG in a dose of 350 g/ha).

The common characteristic in the growth of the crops, which also affects the formation of the studied features, is that the best values were those obtained from the variants treated with Arat - 100 g/ha, Derbi super - 33 g/ha, Sekator OD in a dose of 100 cm^3/ha and Pallas 75- 200 g/ha (variants 2,1,4,8). The other variants showed lower values in a descending order. For most of the features, the values registered in variant № 7 (Axial 050 EK - 600 cm^3/ha) are the same as those of the control sample.

The provedness of the differences between the studied variants regarding the registered yield (kg/ha) in table 3 has been assessed by means of a dispersion analysis (ANOVA method). As with the analyzed features, the lowest values of the yield were registered in the variants treated with Axial 050 EK – 600 cm^3/ha and Laren 20 SG – 30 g/ha, and the highest yield was obtained after applying the herbicide Pacifica WG – 350 g/ha. The data correlates with the specified effectiveness of the herbicidal preparations (table 2).

Table 2. Weed status and herbicide efficiency 30 days after treatment

Weed status (30 days afer treatment: 3-year averages 2012-2014): MC (monocotyledonous) and DC (dicotyledonous) weeds; HE = herbicide efficiency									
Treatment		Annual weeds (AW)			Perennual weeds (PW)			Total AW+PW	HE %
		MC	DC	Total	MC	DC	Total		
		Weeds number per square metter							
1	Derby super	2.0	4.0	6.0	10.0	0.3	10.3	16.3	62.3
2	Arat	1.0	6.7	7.7	7.3	0.7	8.0	15.7	63.7
3	Laren 20 SG	3.3	6.3	9.6	8.7	0.3	9.0	18.6	56.9
4	Secator OD	3.0	6.0	9.0	5.3	-	5.3	14.3	66.9
5	Lintur 70 WG	3.3	6.3	9.6	6.0	0.3	6.3	15.9	63.2
6	Axial 1 + 050 EK	-	5.3	5.3	5.0	1.0	6.0	11.3	73.8
7	Axial 050 EK	-	20.3	20.3	11.7	4.0	15.7	36.0	16.7
8	Pallas 75 WG	-	4.7	4.7	5.3	0.3	5.6	10.3	76.2
9	Pacifica WG	-	4.0	4.0	4.0	1.0	5.0	9.0	79.2
10	Control	4.3	24.3	28.6	8.3	6.3	14.6	43.2	0

Table 3. Significance of the differences to the control for tested properties of wheat (average for the period 2012- 2014)

Treatment		$\bar{x}$	D	Sign.	Treatment		$\bar{x}$	D	Sign.
Height of wheat plant (cm)					**Number of grain in ear of wheat**				
9	Pacifica WG	124.10	13.90	++	9	Pacifica WG	33.25	8.82	+++
2	Arat	123.42	13.22	+	2	Arat	31.75	7.32	++
1	Derby super	123.30	13.10	+	1	Derby super	31.67	7.24	++
4	Secator OD	123.10	12.90	+	4	Secator OD	31.60	7.17	++
8	Pallas 75 WG	122.87	12.67	+	8	Pallas 75 WG	31.55	7.12	+
6	Axial 1+050 EK	122.68	12.48	n.s	6	Axial 1+050 EK	31.40	6.97	+
3	Laren 20 SG	122.45	12.25	n.s	5	Lintur 70 WG	31.10	6.67	+
5	Lintur 70 WG	122.22	12.02	n.s	3	Laren 20 SG	30.40	5.97	+
7	Axial 050 EK	116.32	6.12	n.s	7	Axial 050 EK	28.10	3.67	n.s
10	Control	110.20			10	Control	24.43		
	LSD	5%	1%	1%		LSD	5%	1%	1%
		12.5	13.75	14.23			5.45	7.15	8.45
Weight of grain (g)					**Grain yield (kg ha^{-1})**				
9	Pacifica WG	1.75	0.54	+++	9	Pacifica WG	521.4	169.2	+++
4	Secator OD	1.74	0.53	+++	4	Secator OD	520.0	167.8	+++
2	Arat	1.69	0.48	+++	6	Axial 1+050 EK	518.6	166.4	+++
1	Derby super	1.68	0.47	+++	8	Pallas 75 WG	515.3	163.1	+++
8	Pallas 75 WG	1.67	0.46	+++	1	Derby super	508.2	156.0	+++
5	Lintur 70 WG	1.67	0.46	+++	5	Lintur 70 WG	493.5	141.3	+++
6	Axial 1+050 EK	1.66	0.45	++	2	Arat	482.8	130.6	+++
3	Laren 20 SG	1.62	0.41	++	3	Laren 20 SG	466.5	114.3	+
7	Axial 050 EK	1.42	0.21	n.s	7	Axial 050 EK	406.7	54.5	n.s
10	Control	1.21			10	Control	352.2		
	LSD	5%	1%	1%		LSD	5%	1%	1%
		0.22	0.39	0.45			48.67	96.83	111.56

Conclusions

During the period of the experiment, it was established that the most efficient herbicides in the fight against the weeds in the winter wheat are Pacifica WG in a dose of 350 g/ha (79.2%), Pallas 75 WG in a dose of 200 g/ha (76.2%) and Axial 1 plus 050 EK - 1000 cm3/ha (73.8%). They control 100% of the weeds *Avena fatua L.* and *Alopecurus myosuroides Huds.* The weakest is the effect of the herbicide Axial 050 EK in a dose of 600 cm^3/ha (16.7%), because it controls only the monocotyledonous weeds and their density among the crops is low.

The highest values regarding the number of grains per ear, the weight of the grains on the ear and the yield were registered in variant № 9 (Pacifica WG in a dose of 350 g/ha) followed by Sekator OD in a dose of 100 cm^3/ha, Arat - 100 g/ha, Pallas 75 WG in a dose of 200 g/ha and Axial 1 plus 050 EK - 1000 cm3/ha as the differences with the untreated control sample (№ 10) have been statistically proven.

No differences have been proven between the separate herbicides regarding the yield, with the exception of variant 7 (Axial 050 EK in a dose of 600 cm^3/ha).

By eliminating the competition of the weeds, the herbicides have a favourable effect on the height of the wheat plants.

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PHENOLOGICAL CHARACTERISTICS OF SOME CULTIVARS OF WINTER OATS UNDER THE CONDITIONS OF CENTRAL SOTHERN BULGARIA

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Abstract

Within the period 2006-2009 in the experimental field of the Department for Plant Production at the Agricultural University – Plovdiv, Bulgaria, we conducted a field experiment for the purpose of following and comparing the duration of the inter-phase periods for three winter genotypes of oats (cv. Dunav 1, cv. Resor 1 and line M-K) and also to establish the correlative dependence on the quantity of the rainfall during the respective periods. The sowing was conducted in mid-October based on the split plot design method in four repetitions over an area of 10,5 m^2 and a sowing rate of 500 k.s./m^2 (kernel seeds) after previously grown sunflower. The statistical processing of the experimental data has been made using SPSS V.9.0 for Microsoft Windows.

It has been established that the line M-K develops faster by the third-leaf stage and earlier enters the stage of panicle emergence but its period from panicle emergence to full maturity is longer. The vegetation period is 3-6 days longer compared with Dunav 1 and Resor 1 which develop in parallel. For the tested cultivars, the duration of the periods till germination and the third-leaf stage is in significant positive dependence on the registered rainfall. The larger quantity of the rainfall shortens the period from stem elongation to the panicle emergence for line M-K and Dunav 1 but this dependence was not registered for cv. Resor 1. For all tested genotypes, the quantity of the rainfall within the period from panicle emergence to the full maturity stage and the duration of the period are in a state of significant positive dependence (R=0,874 to 0,962).

Keywords: *winter oat, phenological stages, rainfall, correlative coefficients*

Introduction

The production potential of the crops is to a large extent a function of the combination of agro-meteorological factors in each year. The changes in the climate over the last few years are mostly related to the increase of the temperatures and the reduction of the water resources. In their in-depth survey Porter and Semenov (2005) analyzed the reaction of the main crops towards the climatic changes. They emphasized that temperature rise and significant rainfall variation decreased the chance of obtaining high yield.

A number of surveys have been aimed at establishing the influence and mechanisms by which climate change might affect crop and food quality, for either human or animal nutrition. Crop quality is a multi-faceted and complex subject involving growth, storage and processing pre- and post-harvest, and including nutritional, technological and environmental aspects (Kettlewell *et al.*, 1999; Gooding *et al.*, 2003; Martre *et al.* 2003). There are Bulgarian authors who are also looking for dependence and are trying to explain the influence of the main agro-meteorological factors on the yield and the quality of the field crops (Georgieva, 1996; Georgieva & Kostov, 1996)

The oats are a crop which is very sensitive to high temperatures during the summer and low temperatures during the winter. In addition, it is very demanding with reference to the rainfall during the vegetation period. In relation to this, it is crucial to be familiar with the reaction of each cultivar to these factors, which can be of use when deciding on their agrotechnology

(Georgieva,1996). The purpose of the conducted observations and analyses was to establish some basic dependence between the duration of the interphase periods and the quantity of the registered rainfall under the conditions of Central Southern Bulgaria.

Materials and Methods

Within the period 2006-2009 in the experimental field of the Department of Plant Production at the Agricultural University – Plovdiv a field experiment has been conducted on soil of the type Mollic Fluvisols based on FAO (Popova end Sevov, 2012). The development was followed by comparing the duration of the inter-phase periods (sowing – emergence (SE); emergence-3rd leaf (E3); 3rd leaf -tillering (3T); tillering -stem elongation (TS); stem elongation -panicle emergence (SP); panicle emergence – full maturity (PF) and growing season (GS) of three winter oat cultivars – Dunav 1, Resor 1 and line M-K. The correlative dependence between the periods was established, as well as the dependence of each period on the amount of rainfall.

The sowing was conducted in mid-October based on the split plot design method in four repetitions over an area of 10,5 m^2 and sowing rate of 500 kernel seeds/m^2 after previously grown sunflower. The fertilization rate used was N- 60 kg/ha, P – 80 kg/ha and K – 80 kg/ha. The statistical processing of the experimental data has been made using SPSS V.9.0 for Microsoft Windows (SAS Institute Inc.1999).

The combination of the meteorological factors within the survey period varied which determined the years of the yield as specific with reference to the agro-meteorological conditions for growing the crop. The first year of the experiment 2006/2007 started with favorable temperatures. The average temperatures from October 2006 to March 2007 were 2 to 12º C higher than those in the long term. At the same time, we registered extremely low values of the rainfall in that period, which compromises the germination and the optimal development of the plants. The limited quantities of the rainfall in combination with the high temperatures in March and April shorten the phenological stages which determine the number and the size of the reproductive organs of the panicle of the tested genotypes. Although the better conditions for the oats occurred later, they could not influence significantly the formation of the yield. This made the vegetation year 2006/2007 unfavourable for the optimal development of the winter oats.

The vegetation period 2007-2008 was the most favorable for the development of the oats. The temperatures in October and November 2007 were close to normal, but the heavy precipitations (115 mm/m^2 higher the norm) impeded the sowing within optimal terms. However, the favorable temperatures during the following period allowed the germination of 97% of the sown seeds and their survival in winter. The favorable temperatures and the high values of the rainfall evenly distributed in May and June from the panicle- emergence to the full-maturity stage resulted in the optimal formation of grains on the panicle.

During the vegetation year 2008-2009, the winter was mild, with temperatures close to the norm in spring and there were frequent heavy precipitations. This allowed the normal development of the plants. However, later in April, May and June, the rainfall was insufficient (81,2 mm/m^2) and significantly below norm (107 mm/m^2), which substantially impeded the formation and the optimal development of the components of the panicle.

Results and Discussion

Cultivar Dunav 1. Cultivar Dunav 1 is characterized by its flexibility and adaptability to the various agro-climatic conditions. Table 1 shows that even when sowing 2-3 weeks later than the optimal term in 2007, the vegetation period for the year 2007/2008 was only 4 days longer than the vegetation period in 2006/2007. The sowing in 2008 was conducted during the most favorable period but despite this, the vegetation period in 2008/2009 was the longest. There is

no proven relation between the optimal period of sowing and the duration of the vegetation period.

We discovered (Table 2) strong positive dependence between the duration of the emergence and the period from the emergence to the formation of the third leaf and also between both periods and the period extending from the formation of the third leaf to the tillering stage. The duration of these periods is in strong positive dependence on the precipitations during that period. Between the duration of the inter-phase period from tillering to the stem elongation stage and the duration of the previous stages there is strong negative dependence (R= -0,866 до R= -0,965). The registered precipitations had a positive influence on the duration of the periods of sowing till germination, from the tillering stage to the stem elongation stage and from the period of panicle emergence to full maturity but the influence on the duration of the period from tillering to stem elongation is negative. The duration of the period from panicle formation to full maturity manifests strong positive correlative dependence on the quantity of the registered rainfall within this interval since the vegetation period (R= 0,890). The running of this inter-phase period does not depend on the total quantity of the rainfall during the vegetation period.

Table 1. Duration of inter-phase periods (number of days) and amount of rainfall (mm/m^2) in the respective period, cultivar Dunav 1

Inter-phase period	SE	E3	3T	TS	SP	PF	GS
2006/2007							
Duration	13	18	6	91	36	49	200
Rainfall	61,5	6,0	7,7	122,7	25,6	262,4	355,9
2007/2008							
Duration	33	73	11	44	30	46	204
Rainfall	147,7	93,1	2,5	41,2	29,5	98,5	412,5
2008/2009							
Duration	10	8	3	163	29	37	240
Rainfall	13,9	0,0	4,2	293,2	41,5	17,5	370,3

Table 2. Correlations between the duration of inter-phase periods (number of days) and the amount of rainfall (mm/m^2) (R), cultivar Dunav 1

	SE	E3	3T	TS	SP	PF	GS	R SE	R E3	R 3T	R TS	R SP	R PF	R GS
SE	1,000	0,998	0,966	-0,866	-0,268	0,391	-0,525	0,972	0,998	-0,667	-0,823	-0,395	-0,073	0,933
E3		1,00	0,972	-0,877	-0,245	0,412	-0,545	0,977	0,996	-0,649	-0,836	-0,416	-0,050	0,925
3T			1,00	-0,965	-0,011	0,614	-0,726	0,998	0,948	-0,453	-0,941	-0,618	0,186	0,809
TS				1,000	-0,250	-0,799	0,881	-0,959	-0,833	0,204	0,997	0,802	-0,436	0,628
SP					1,000	0,782	-0,679	-0,033	-0,327	0,897	-0,327	-0,779	0,980	0,596
PF						1,000	-0,989	0,597	0,332	0,426	-0,845	-0.998	0,890	0,034
GS							1,000	-0,711	-0,471	-0,284	0,916	-0,989	-0,811	-0,184
R SE								1,000	0,955	-0,472	-0,934	-0,601	0,165	0,822
R E3									1,000	-0,712	-0,786	-0,337	-0,135	0,954
R 3T										1,000	0,125	-0,421	0,792	-0,890
R TS											1,000	-0,847	-0,507	-0,564
R SP												1,000	-0,887	-0,039
R PF													1,000	-0,426
RGS														1,000

Cultivar Resor 1. This cultivar is of interest to us because it has been determined as a reference for quality in Bulgaria. The duration of the inter-phase periods and the conditions under which they develop affect the quality and the determination of the dependence explains to a large extent the obtained results. The stages of tillering and in particular stem elongation

are crucial for the formation of the future reproductive organs of the panicles. For that reason, the good knowledge of this dependence would help to plan the optimal period for sowing and the cares for the plants. The duration of the periods from tillering to stem elongation and from stem elongation to panicle emergence (Table 3) are in reverse dependence on the period from panicle emergence to full maturity but also in strong positive dependence on the duration of the vegetation period.

Table 3. Duration of inter-phase periods (number of days) and amount of rainfall (mm/m^2) in the respective period, cultivar Resor 1

Inter-phase period	SE	E3	3T	TS	SP	PF	GS
2006/2007							
Duration	13	18	6	91	35	50	200
Rainfall	61,5	6,0	7,7	122,7	25,6	262,4	455,9
2007/ 2008							
Duration	33	73	11	44	30	44	202
Rainfall	147,7	93,1	2,5	41,2	29,5	98,5	412,5
2008/ 2009							
Duration	10	8	3	163	30	36	240
Rainfall	13,9	0,0	4,2	293,2	41,5	17,5	370,3

Table 4. Correlations between the duration of inter-phase periods (number of days) and the amount of rainfall (mm/m^2) (R), cultivar Resor 1

	SE	E3	3T	TS	SP	PF	GS	R SE	R E3	R 3T	R TS	R SP	R PF	R GS
SE	1,000	0,998	0,966	-0,866	-0,943	0,201	-0,564	0,972	0,998	-0,667	-0,823	-0,395	-0,073	0,112
E3		1,00	0,972	-0,877	-0,950	0,224	-0,583	0,977	0,996	-0,649	-0,836	-0,416	-0,050	0,135
3T			1,00	-0,965	-0,997	0,446	-0,757	0,998	0,948	-0,453	-0,941	-0,618	0,186	0,364
TS				1,000	-0,983	-0,664	0,902	-0,959	-0,833	0,204	0,997	0,802	-0,436	-0,594
SP					1,000	-0,516	0,807	-0,995	-0,920	0,381	0,965	0,678	-0,263	-0,436
PF						1,000	-0,922	0,427	0,139	0,596	-0,722	-0,979	0,962	0,996
GS							1,000	-0,743	-0,511	-0,239	0,933	0,981	-0,782	-0,884
R SE								1,000	0,955	-0,472	-0,934	-0,601	0,165	0,343
R E3									1,000	-0,712	-0,786	-0,337	-0,135	0,049
R 3T										1,000	0,125	-0,421	0,792	0,666
R TS											1,000	0,847	-0,507	-0,657
R SP												1,000	-0,887	-0,957
R PF													1,000	0,983
RGS														1,000

For example, very strong positive relations were established between the duration of the period from sowing to germination, from germination to the third-leaf formation and from the third-leaf formation to tillering. They all are in negative dependence on thefollowing two periods – from tillering to stem elongation and from stem elongation to panicle emergence.
The periods from sowing to the third-leaf formation largely depend on the registered precipitations. The established dependence once again confirms the restrictive influence of the rainfall on the determination of the sowing time and the initial development of the oats plants. The larger quantity of the rainfall extends the periods from the third-leaf formation to tillering. They have even stronger negative influence on the period from tillering to stem elongation. Between the duration of the entire vegetation period and the quantity of the registered rainfall there is strong negative dependence (R=-0,884). The longest vegetation period was registered in 2009 – 240 days during which the quantity of the rainfall was the smallest (370 ml/da). All of this once again proves that the total quantity of the rainfall, its distribution over the vegetation period is crucial for the duration of the separate inter-phase periods and the latter affect the duration of the vegetation. The total quantity of the registered

rainfall extended the period from tillering to stem-elongation in 2009 (Table 4) but significantly shortened the period from panicle emergence to full maturity.

Line M-K. The line has been selected at the Department for Plant-growing at the Agricultural University and is considered to be promising in terms of yield and quality indices. In its development there are some differences in the running of certain stages. For example, the line germinates in a shorter period of time (two to three days) but it takes longer for the line to reach the third-leaf formation and tillering stages. In spring the development is again faster as it enters the stem elongation and the panicle emergence stages earlier than the other two tested cultivars (Table 5).

Table 5. Duration of inter-phase periods (number of days) and amount of rainfall (mm /m^2), in the respective period, Line M-K

Inter-phase period	SE	E3	3T	TS	SP	PF	GS
2006/ 2007							
Duration	10	21	9	88	33	54	205
Rainfall	59,5	15,7	0,0	122,7	15,3	272,7	485,9
2007/ 2008							
Duration	31	75	14	38	30	51	208
Rainfall	146,9	93,1	2,5	41,2	29,5	98,8	412,0
2008/ 2009							
Duration	10	8	5	159	29	41	243
Rainfall	0,0	0,0	4,2	280,4	28,0	17,5	330,1

The results from the conducted correlative analysis (Table 6) give us additional information on the dependence between the duration of the interphase periods and the registered rainfall. The data shows that between the periods from sowing to germination, from germination to the third-leaf formation and from the third-leaf formation to tillering there is strong positive dependence. The duration of the same interphase periods is in negative dependence on the duration of the period from tillering to stem elongation. The extension of the period from tillering to stem elongation affects the duration of the entire vegetation period.
The period from panicle emergence to full maturity, in which the grains are formed and their quality is determined, is in negative dependence on the duration of the period from tillering to stem elongation.
The precipitations extend the period of germination and the third-leaf formation as well as the duration of the period from tillering to stem elongation and from panicle elongation to full maturity. The registered larger quantity of the rainfall during the stem elongation period accelerates the entering of the plants into the panicle emergence stage (R=-0,943). The duration of the vegetation period is in negative dependence on the registered rainfall.

Table 6. Correlations between the duration of inter-phase period (number of days) and the amount of rainfall (mm/m^2) (R), Line M-K

	SE	E3	3T	TS	SP	PF	GS	R SE	R E3	R 3T	R TS	R SP	R PF	R GS
SE	1,000	0,956	0,839	-0,740	-0,386	0,185	-0,331	0,863	0,963	0,223	-0,682	0,670	-0,316	-0,082
E3		1,00	0,962	-0,905	-0,097	0,467	-0,594	0,974	0,998	-0,074	-0,867	0,422	-0,023	0,216
3T			1,00	-0,987	-0,178	0,690	-0,791	0,999*	0,955	-0,343	-0,970	0,160	0,250	0,473
TS				1,000	-0,120	-0,799	0,880	-0,978	-0,894	0,492	0,997	0,004	-0,405	-0,611
SP					1,000	0,835	-0,743	0,133	-0,123	-0,985	-0,412	-0,943	0,997	0,951
PF						1,000	-0,989	0,656	0,444	-0,917	-0,845	-0,605	0,874	0,964
GS							1,000	0,762	-0,573	0,846	0,916	0,478	-0,791	-0,913
R SE								1,000	0,967	-0,300	-0,958	0,204	0,206	0,433
R E3									1,000	-0,049	-0,854	0,445	-0,048	0,190
R 3T										1,000	0,561	0,873	-0,995	-0,990
R TS											1,000	0,086	-0,479	-0,673
R SP												1,000	-0,916	-0,794
R PF													1,000	0,971
RGS														1,000

Conclusions

The tested cultivars show certain specific features in their phonological development and the duration of the interphase periods. Line M-K develops faster by the third-leaf formation and earlier enters the panicle emergence stage but the period from panicle emergence to full maturity is longer. The vegetation period is 3-6 days longer compared with Dunav 1 and Resor 1, which develop in parallel.

Between the periods from sowing to germination, from germination to the third-leaf formation and from the third-leaf formation to tillering there is strong positive correlative dependence. However, their duration is in negative dependence on the duration of the stage of tillering and stem elomgation. We have established strong positive correlative dependence between the duration of the period from stem elongation to panicle emergence and the period from panicle emergence to full maturity for cultivar Dunav 1 and line M-K and negative dependence for cultivar Resor 1.

The correlative dependence between the quantity of the registered rainfall and the duration of the inter-phase periods shows that for the tested cultivars the duration of the periods from germination to third-leaf formation has strong positive dependence on the registered rainfall. The larger quantity of the rainfall shortens the period from stem elongation to panicle emergence for line M-K and Dunav 1 but this dependence has not been registered for cultivar Resor 1. For all tested genotypes the quantity of the registered rainfall within the period from panicle emergence to full maturity and the duration of the period are in strong positive dependence (R=0,874 to 0,962).

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FIRST RECORD OF PHOMOPSIS SP. ON CHICK PEA (Cicer arietinum) IN BULGARIA

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Abstract

Genus *Phomopsis* includes saprophytes and pathogens on a large number of plants and can cause several diseases, some of them economically important. This paper presents the results of investigation on the occurrence of *Phomopsis/Diaporthe* sp. on *C. arietinum* in Bulgaria. It describes the symptoms of infection on chickpea, the morphological and cultural characteristics of the pathogen on acid PDA (APDA), the biometrical values and reproductive structures on naturally infected plants as wel on artificial nutrition medium. The isolates produced white to light grayish colonies which grew fast and filled the petri dishes for 6-7 days. The mycelium later turned dark, gray-greenish and finally brownish-black. The stroma was black, with irregular shape and size, and pycnidia was rarely observed in it. The pathogen formed two types of conidia - α и β. The α-conidia were unicellular, elliptical, hyaline, containing 2 – 4 fatty cells, although most often two. The β-conidia were observed more rarely. They were unicellular, filamentous, hyaline, straight or slightly curved. Pycnidia with α- and β- conidia were later formed in the infected tissues. Our results showed that the cultural and morphological characteristics of the isolated pathogen coincided with the description of *Phomopsis / Diaporthe* sp. This investigation is the first record of the *C. arietinum* as a host of *Phomopsis / Diaporthe* sp. in Bulgaria.

Key words: *Phomopsis/Diaporthe* sp., chickpea ,*Cicer arietinum*, first record

Introduction

Genus *Phomopsis* includes saprophytes and pathogens on a large number of plants and can several diseases, some of them economically important. The species from this genus are mainly described on the basis of their hosts, and to a lesser degree on the basis of the morphological and cultural characteristics, as well as the biometrics, the anamorph and teleomorph structures. The investigations, however, have revealed that the relation with the host has little taxonomic significance due to the wide range of hosts for some species (Santos and Phillips, 2009). Such species are *Phomopsis longicola* and *Diaporthe phaseolorum* var. *sojae*, which were isolated from *Glycine max* (Gomes et al. 2013: Zhang et al. 1997; Santos et al. 2011), *Abutilon theophrasti* (Li et al. 2001; Vrandecic et al. 2007), *Arachys hypogaea* (Sanogo & Etarock, 2009), *Capsicum annum* (Pennycock, 1989), *Cucumis* melo (Zhang et al. 1997), *Lycopersicum esculentum, Phaseolus acutifolius, Ph. lunatus, Ph. vulgaris, Pisum sativum, Trifolium pratense, Vigna unguiculata* (Aćimović M., 1988; Malvick, 1997; http://cropgenebank.sgrp.cgiar.org). Additionally, several phomopsis species can attack a single plant species. On the other hand, the morphological characteristics are not always suitable for specific identification due to their plasticity and concurrence with the other species.

In this paper we presented the results regarding investigation on the occurrence and first record of *Phomopsis/Diaporthe* sp. on *C. arietinum* in Bulgaria.

Material and method

In July 2011 and 2012 a field survey was done on the experimental *C. arietinum* crops in Dobrudzha Agricultural Institute – General Toshevo. Presence of symptomatis plants were observed and samples taken for further analyzis. The chickpea stems were washed with running water and 5 mm pieces of it were cut and disinfected in 1 % solution of NaOCl for 3 min. After they were triple washed with distilled water, left to dry on filter paper and placed in petri dishes (ø 90 mm) with acid PDA (APDA). The petri dishes were incubated in thermostat, in dark, at 25°C. Pathogen was isolated by direct transfer of pycnidia and exsudate of conidia on APDA. The pure cultures were kept in thermostat for 45 days and continuously observed for growth and spore formation. The diameter of pycnidia and perithecia was measured, as well as the length and width of pycnidiospores, asci and ascospores by light microscopy. The ascospores were spread on water agar and subsequently transferred one by one on APDA to confirm their anamorph form.

To check the pathogenicity of the fungus species, two-week chickpea plants (cultivar Balkan) were used. Tips of plants were cut off above the second internode. Mycelium discs were excised from the ends of the 5-day cultures using plastic straws about 3 cm long and directly transfered on the cut plants. The inoculated plants were kept in a greenhouse at 25^{o} C and RH 90-95 %. After three weeks, the size of lesion on inoculated plants was measured. Plants, on which PDA disks without the pathogen were placed, were used for control.

Results and discussion

Our observations showed that the typical symptoms of infected plants were dry rot, with lighter areas around the stem nodes and the petioles, which caused wilting or death of the plants. Usually these lesions were not surrounded with stripes. Small black pycnidia dipped in the epidermis were found on the main stem and the branches (Fig. 1). The pycnidia were most numerous at the base of the main stem, around the nodes, and less on edges of lesions. Also, V-shaped necrotic spots were found on the leaves, beginning from the tip downwards along the main nerve.

The isolates produced white to light grey colonies which grew up quickly and filled the petri dishes for 6-7 days. The mycelium later turned dark, grey-greenish and finally brownish-black. The mycelium was branched and septate. The stroma was black, of irregular shape and size and pycnidia were rarely observed in it. The pathogen formed two types of conidia – α and β (Fig. 2, 3). After placing infected plant parts on moist filter paper, in a next few days white drops of α-conidia were detected to emerge from the pycnidia ostiole (Fig. 4). The size of the pycnidia was 120 – 298 x 95 - 202 μm. The α-conidia were unicellular, elliptical, hyaline and containing from 2 to 4 fatty cells, althoug most often two.. The size of the α-conidia was 4.5 – 10.0 μm x 2.0 – 3.5 μm. The β-conidia were observed less frequently. They were unicellular, filamentous, hyaline, straight or slightly curved.

The symptoms which cause on infected plants, cultural and morphological characteristics of the isolated pathogen allowed referring it to genus *Phomopsis* (Aćimović, 1988; Costamilan et al. 2008).

Both, pycnidia and perithecia were observed on over wintering infected plant parts (Fig. 5). The perithecia had protruded elongated tips and size 185 – 350 μm x 150 – 310 μm. The size of asci was 27 – 58 μm x 5.0 – 10.3 μm (Fig. 6). The ascospores were two-cellular, hyaline, elongated to elliptical, clamped at the septum, with size 8.5 – 13.5 μm x 3.0 – 4.5 μm.

The morphological characteristics and biometrical values of the reproductive structures of isolated fungus corresponded to the description of *Diaporthe* sp. (Aćimović, 1988; Vrandecic et al. 2005; Grijalba and Guillin, 2007).

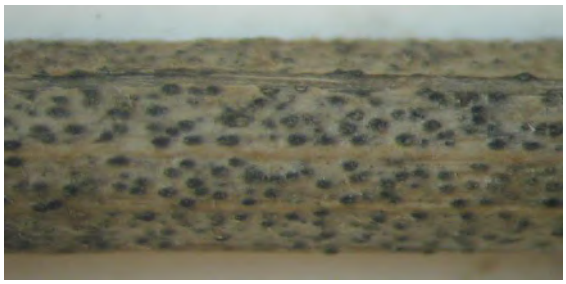

Figure 1. Chickpea stems: symptoms of *Phomopsis*

Figure 2. Phomopsis sp.: α- conidia

Figure 3. Phomopsis sp : β- conidia

Figure 4. Pycnidia and leaking of spore mass

Figure 5. Perithecia on a stem of a naturally infected chickpea plant

Figure 6. Asci with ascospores of *Phomopsis*

After three weeks of inoculation passed, the infected plants showed brownish coloration of stems, and the length of the lesions varied from 5 to 30 mm. The symptoms on the artificially

infected plants were the same as those naturally infected. The necrotic tissue grew upwards and downwards from the place of infection. Pycnidia with α- and β- conidia were later formed in the infected tissues. On the control plants lesions were not observed. To confirm the pathogen identification, re-isolation from lesions on artificially infected plants was done.

Conclusion

Based on the results from this investigation, symptoms, cultural and morphological characteristics of the isolates from chickpea, it can be concluded that isolated fungus belongs to *Phomopsis / Diaporthe* sp.

Our results aslo present the first record of chickpea as a host of *Phomopsis / Diaporthe* in Bulgaria.

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MODELING THE PRODUCTION AND THE PROTECTION OF THE TOMATO CROP IN MIDDLE EGYPT

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Abstract

In order to create successful program for the integrated management of pests in tomato, the simulation's software was used in this study as tools for the pest management. The used program begins with series of screens that enables the user to modify any of the default data at the beginning of the simulation. The key data that required for starting simulation are: tomatoes varieties, dates of cultivations, the geographical location, and the climatic data. Essential data for insects that required for starting the simulation are: insects' life tables, numbers of individuals in the examined samples and climatic data. Actual growth curve for tomatoes, and the predicted by the forecast model as expressed it through average deviations from averages of vegetative growth phenomena such as dates of agriculture, vegetation growth, blooming, formation of fruits, and the fruits ripening were compared. Results showed the average of deviation as follows: 0.00,-2.33-2.44,-4.00 and 13, 44 days respectively for the planting dates, vegetative growth, blooming, formation of fruits, and the fruits ripening. The Differences between expected and actual numbers in the numerical density of insects on tomato were tested also. The model explained the level of accuracy in predicting the density of pests in three seasons (2011-2013): 66.35% of aphid, 68.62% for red spider mite, 82.78% of white fly, 64, 11% cotton leaf worm. 73.96% for potatoes tuber moth, 56.43%, for tomato fruit worm, 50, 83% tobacco bud worm. Results indicated that the actual growth's curves not much different from those obtained by the laboratory model and can be implemented as a tool of integrated production and the protection for protection for tomato.

Key words: *Tomatoes, modeling, protection, prediction, middle Egypt.*

Introduction

Prediction is the expectation of a particular act in the future and prediction of plant pests is the expectation of the occurrence of such lesions early, before it takes place with the appropriate period of time. This critical period can be taken where necessary to resist the disease, and thus avoid the heavy loss caused by pests. In other words, the prediction of plant pests explain to farmers in a particular area that the conditions are right enough for the disease and that the use of effective means to resist the disease would lead to the survival of crop injury and consequently a large profit to the farmer.

Despite many Integrated Pest Management (IPM) successes using technologies such as host plant resistance and biological control, synthetic pesticides have remained the predominant component of IPM programs through the last three decades (Allen and Rajotte, 1990). Wearing (1988) has pointed out that the delay in the adoption of IPM has to do with the complexity of the programs and the considerable time and resources that must be devoted to implementation.

Despite the complexity, looking at pest management from the whole-farm level opens new avenues for pesticide and pest management (Stone and Warren, 1993). By anticipating how the overall farm plan contributes to pest outbreaks, one can avoid cropping systems and practices that lead to pest management problems. To help manage the complexity of this process, a multi-objective computer decision aid was designed to help farmers generate

alternative whole-farm plans that are profitable and environmentally sound (Buick *et al.*, 1992: Stone 1995).

A decision support system (DSS) is a computerized system for accessing and processing data and providing recommended courses of action as developed through the use of analytical methods (Ignizio 1991). In agriculture, a DSS may help farmers make tactical and strategic management decisions in areas such as cultivar selection, timing of planting and harvesting, and pest management (Plant and Stone, 1991).

The crops system is a whole-farm planning and scheduling system that considers soil conservation, nutrient management, pest management, and economic constraints to generate farm plans (Buick *et al.*, 1992). Each of the areas of consideration requires an automated evaluator to provide input for the process of assessing potential farm plans. This research deals specifically with the pest management aspect of this system.

In Egypt Tomatoes are subject to attack by a large number of insect pests from the time plants first emerge in the seed bed until harvest, however, severe damage may result either from their feeding on the fruit or by spreading certain diseases .i.e. Cutworm *Agrotis ipsilon* (BCW), tomatoes' whitefly *Bemisia tabaci* (CWF), tomatoes' aphid *Aphis gossypii* (CA), green peach aphid *Myzuz persicae* (GPA) , Potato tuber moth *Phthorimaea operculella* (PTM), tomatoes' leaf worm *Spodoptera Littoralis* (CLW) , Faba bean leaf miner *Liriomyza trifolii* (FBLM) , two spotted spider mite *Tetranychus urticae* (TSSM), tomatoes fruit worms *Helicoverpa zea*,(TFW), and tobacco budworm, *Heliothis virescens* (TBW).

Climate has a profound effect on the distribution and abundance of invertebrates such as insects, and the mathematical description of the climatic influence on insect development has been of considerable interest among entomologists. Additionally, as temperature exerts great influence among the climate variables, by directly affecting insect phenology and distribution, most of the models that describe insect development are temperature driven.

The rates of development in insects under natural conditions are largely determined by temperature. In most microenvironments, temperature is characterized by daily and seasonal cyclic variations with superimposed irregular fluctuations. However, studies of insect development rate most often involve experiments performed under constant temperatures (Howe 1967). In the development and application of development-rate models, it is always assumed that development rate at a given temperature is independent of thermal regime, whether the model is linear or nonlinear in relation to temperature. This assumption is also inherent in efforts to derive development-rate models from data obtained under varying temperatures, such as the work by Dallwitz & Higgins (1978). According to this assumption, development rate follows a definite function with respect to temperature, when other factors are equal and the amount of development can be calculated by ac-cumulating the fraction of development per unit time; i.e., rate summation (Kaufmann 1932). The procedure may be expressed as:

$$D = f\, r\,[T\,(t)]\,DT$$

Where development D: is a function of temperature. T: which in turn is a function of time, t, and the development rate, r; adjusts instantaneously to temperature.

The above assumption is fundamental to the formulation of development-rate functions for phonological models. Life table studies are fundamental to not only demography but also too general biology. In such studies, development tunes and survival rates of each stage, longevity of adults, and the daily fecundity of females are recorded for every individual. Using elementary statistics, means and standard deviations can be calculated. In traditional life-table analysis, these means are used to calculate age-specific survival rates and age-specific fecundity using either the Leslie matrix (Leslie 1945) or Birch's method (Birch 1948). These procedures have been widely used by researchers in many different fields (Laing 1969, Shib *et al.* 1976, Cave & Gutierrez 1983, Vargas *et al.* 1984, Carey & Vargas 1985). The number

of days between observable events, such as tomatoes' seedling emergence and first squares of the duration of insect generations can characterize the growth and development of plants and insects. The number of days between events, however, may be misleading because growth rates vary with temperatures. The measurement of events can be improved by expressing development units in terms of the temperature and time. The deviation between events is then based on accumulated degrees per unit time above a lower temperature re-presenting a threshold of growth.

The goal of this study is to test the accuracy of the "Model Cottamin” (under publication) in measuring the interaction of pest-plant-weather components on two tomatoes' varieties in forecasting the occurrence timing of plant phenomena's and pests infestation’s peak as well as the density of its populations.

Materials and methods

COTTAMIN **(under publication)** is method derived from temperature for measuring the organisms' growth based on cumulative heat above the lower growth threshold**.** (Wilson and Barnett 1983).

It is consisting of two modules: one for plant phenomena's and the second for the pest's populations. The crop module predicts the four main plant organs; seedlings, leaves, flowers, and bolls. Each component has its own life table measurements such as birth rates, death rates, and growth rates. Pest's module also has the same features for the five tested pests

COTTAMIN validation data were collected from Fayum Governorate tomatoes' fields (90 Km south Cairo) in Middle Egypt.

Data were collected throughout three successive Tomatoes-growing seasons (2011-2013). Hybrid 737 and Kassel Rock varieties cultivated. Data was collected on: 1-pests numbers 2-injured plant numbers, 3-tometoes fruits numbers, 4- predator numbers, and 5-current weather's data.

Protocol was as follows: 1- pheromone traps: Seven BCW, TBW, TFW, PTM and CLW pheromone delta traps were placed from January 1st to the end of December.

The lure baits were replaced with new one every 2 weeks or less depending on the weather. Adult males from each pheromone trap were checked twice per week.

Yellow sticky traps were used for aphids and white fly. Visual examination for red mite was made on all plants found in 25 m quadrate randomly selected.

2- Tomatoes' plants: Twenty-five meters of canopy were examined weekly for:

a) Plant height/m b) Crop injury (serpentine mines in leaves, and holes are chewed in fruits and buds and the infestation percentages were estimated in the lab

Results and discussion

Knowledge acquisition is the process of collecting the knowledge necessary for solving a problem and encoding it into a form that allows for efficient computer manipulation. Knowledge acquisition is comprised of two tasks: knowledge elicitation and knowledge representation. These two tasks do not necessarily occur in sequence and both usually take place throughout the development life cycle as deficiencies in the knowledge base are realized and modifications are made. In knowledge elicitation, domain knowledge is obtained through various means including interviews with experts and book and journal references. In knowledge representation, the elicited knowledge is converted to a form for efficient computer manipulation (Nikolopoulos 1997).

I - Prediction of Tomatoes phenomena's

In 2011 tomatoes' growing season data were collected from three different planting dates in Fayum Governorate cultivated with tomatoes Hybird 737. The three planting dates were: 20/07/2011, 12/08/2011, and 27/08/2011. In 2012 tomatoes' growing season the cultivated

tomatoes' variety was Kassel Rock and the Planting dates were: 15/07/2012, 01/08/2012, and 24/08/2012. The Cultivation Dates for 2013 tomatoes' growing season for Hybrid 737variety were: 28/05/2013, 03/07/2013, and 26/08/2013.

Table (1) illustrated the plant phenomena's; seedling date, 5th vegetative node to 18th node, flowering, fruit formation and fruit ripening. The table shows deviation between the observed data that collected from the fields and the predicted that obtained after applying the Cottamin model by days.

As it could be seen the average of deviation ranged from 0.00, -2.33, -2.44, -4.11, and 13.44 days for; seedling date, 5th vegetative node to 18th node, flowering, fruit formation and fruit ripening respectively.

As it could be concluded from the obtained results, the growth development curves observed actually did not differ significantly than those obtained by COTTAMIN model **(under publication)** as all observed curves demonstrated a similar growth rate curve prior to the COTTAMIN model.

Data in Table (2) demonstrate the deviation of means between observed and predicted tomatoes' phenology by means of heat units.

The careful examination of date in the table reveal that the general average of devotion of DD’s for the nine cultivation dates for the five plant stages tested ranged from 14.40, -2.00, 4.04, -8.22, -5.88 seedling date, 5th vegetative node to 18th node, flowering, fruit formation and fruit ripening respectively.

II – Prediction of Tomatoes' key pests

The proposed model (COTTAMIN) predicate the occurrence of insect pests, consequently the goals of this work is to make the validation of that model.

Simulated and observed tomatoes tested insect pests densities are depicted in Table (3). COTTAMIN Model explained only 66.15 % of the variability in the field for all the tested insects, 66.35% for aphid as an average for the three seasons, 68.62% for red mite, 82.78 % for the white fly, 64.11 % for the cotton Leafworm and 73.96 % for the potato tuber moth, 56.43% for tomatoes fruit worms and 50.83% for tobacco budworm. The predicted trend of population peaks is closed to the actual population peaks, the general average for pest component as whole reached to 66.15 %.

However, the COTTAMIN model **(under publication)** could be successfully used for tomatoes' insect pests' predictions in Fayum, Middle Egypt.

The first effort for a formal description of the relation between temperature and developmental rate was taken by botanists, to model the effect of temperature on plant growth and development. However, similar modeling procedures extended to most of the poikilothermic organisms, including insects as well. To date, the earliest experiment that related the velocity of insect development and heat, was made by Bonnet (1779) on the study of the reproduction rate of *Aphis evonymi*, F. , while the major assumption and principles that have been brought out by these earlier works, constituted the basis for all future research. Nevertheless, since then, several theoretical and experimental works have been carried out and current progress in entomology, mathematics and computation offers new means in describing the relation of temperature to insect development. Based on such linear relationships, between thermal constants and lower temperature thresholds, for several cold-blooded species, it is suggested that there is an inverse relationship between lower temperature thresholds and the thermal constant associated with latitude and/or habitat that adapts each species also based on the particular morphology and size of the species. For example, size at maturity is a function of the rate and duration of growth, and large size at maturity implies a long generation time and a correspondingly large requirement.

Thus, although simple predictive models have been developed during the last century, the development and broader availability of personal computers in the 70s and 80s resulted in the

rapid development of computer-based models to predict responses of insects in relation to climate.

Table (1) Deviation between observed and predicted tomatoes phonology by Cottamin model, by means of days (2011-2013), Fayum, Middle Egypt

Planting Dates		Seedling	5^{th} vegetative node to 18th node	Blooming	Fruit format -ion	Ripening
20/07/2011	Observed	7/20	8/15	8/17	8/23	10/3
	Predicted	7/20	8/15	8/18	8/26	10/12
	Deviation	0	0	-1	-3	-9
12/08/2011	Observed	8/12	9/7	9/9	9/16	11/3
	Predicted	8/12	9/8	9/12	9/20	11/16
	Deviation	0	-1	-3	-4	-13
27/08/2011	Observed	8/27	9/23	9/25	10/3	12/10
	Predicted	8/27	9/24	9/28	10/7	12/17
	Deviation	0	-1	-3	-4	-7
15/07/2012	Observed	7/15	8/8	8/12	8/18	9/25
	Predicted	7/15	8/8	8/13	8/23	10/6
	Deviation	0	0	-1	-5	-11
01/08/2012	Observed	8/1	8/25	8/29	9/4	10/14
	Predicted	8/1	8/27	8/31	9/8	10/29
	Deviation	0	-2	-2	-4	-15
24/08/2012	Observed	8/24	9/16	9/20	9/26	11/10
	Predicted	8/24	9/21	9/25	10/4	12/10
	Deviation	0	-5	-5	-8	-30
28/05/2013	Observed	5/28	6/25	6/27	7/4	8/12
	Predicted	5/28	6/26	6/27	7/5	8/15
	Deviation	0	1	0	1	3
03/07/2013	Observed	7/3	7/27	7/30	8/7	9/14
	Predicted	7/3	8/1	7/31	8/8	9/21
	Deviation	0	-5	-1	-1	-7
26/08/2013	Observed	8/26	9/18	9/21	9/27	11/12
	Predicted	8/26	9/26	9/27	10/6	12/14
	Deviation	0	-8	-6	-9	-32
Average of Deviation		0.00	-2.33	-2.44	-4.11	-13.44

Table (2) Deviation between observed and predicted tomatoes phonology by Cottamin model, by means of heat units (2011-2013), Fayum, Middle Egypt

Planting Dates		Seedling	5th vegetative node to 18th node	Flowering	Fruit formation	Ripening
20/07/2011	Observed	644	391	464	521	1113
	Predicted	659	385	427	538	1123
	Deviation	-15	6	37	-17	-10
12/08/2011	Observed	619	394	424	528	1118
	Predicted	603	384	436	537	1121
	Deviation	16	10	-12	-9	-3
27/08/2011	Observed	597	395	423	530	1120
	Predicted	538	377	426	530	1123
	Deviation	59	18	-3	0	-3
15/07/2012	Observed	655	373	435	529	1126
	Predicted	638	371	428	541	1128
	Deviation	17	2	6.5	-12	-2
01/08/2012	Observed	648.7	376	434	528	1129
	Predicted	607	380	434	541	1123
	Deviation	41.7	-4	0	-13	5.5
24/08/2012	Observed	556	347	440	542	1129
	Predicted	549	381	421	537	1121
	Deviation	6.9	-34	18.87	5	7.6
28/05/2013	Observed	450	397	424	528	1066
	Predicted	475	410	425	539	1124
	Deviation	-25	-13	-1	-11	-58
03/07/2013	Observed	420	379	424	542	1129
	Predicted	465	415	414	529	1134
	Deviation	45	36	-10	-13	5
26/08/2013	Observed	390	376	427	529	1124
	Predicted	406	415	427	533	1119
	Deviation	-16	-39	0	-4	5
Average of Deviation		14.4	-2	4.04	-8.22	-5.88
± S. D		29.74	23.94	15.31	7.12	20.35

Table (3): Simple correlation of Log observed and predicted curves of some tomatoes insect pests during three successive Tomato growing seasons (2011-2013) at Fayum Governorate, Middle Egypt

Pest	Season	R^2		% Accuracy
		Observed	Predicted	
Aphid	2011	0.0979	0.0599	61.18
	2012	0.0138	0.0111	80.43
	2013	0.0902	0.0518	57.43
	Average			66.35
Red Mite	2011	0.4477	0.3303	73.78
	2012	0.0313	0.0269	85.94
	2013	0.3962	0.1828	46.14
	Average			68.62
Whitefly	2011	0.2264	0.1828	80.74
	2012	0.0078	0.0062	79.49
	2013	0.0185	0.0163	88.11
	Average			82.78
Cotton Leafworm	2011	0.1014	0.0456	44.97
	2012	0.0031	0.0026	83.87
	2013	0.3278	0.2081	63.48
	Average			64.11
Potato tuber moth	2011	0.3204	0.2161	67.45
	2012	0.5419	0.3885	71.69
	2013	0.3964	0.328	82.74
	Average			73.96
Tomatoes fruit worms	2011	0.35	0.2726	77.89
	2012	0.0073	0.0013	17.81
	2013	0.1125	0.0828	73.60
	Average			56.43
Tobacco budworm	2011	0.0072	0.0038	52.78
	2012	0.1556	0.0795	51.09
	2013	0.5472	0.266	48.61
	Average			50.83
General Average				66.15

Conclusion

The results show it is possible to simulate the expertise of integrated pest management with a reasonable degree of accuracy.

It is only with this gathering of disciplines that it will be possible to make holistic choices about pest management in our diverse ecosystems.

This expert system, as a standalone decision support tool, needs more testing before it can be used in the field. While the insect component has reached an acceptable level of performance and is ready for further implementation. In this research all weather events were given equal

chances of occurring throughout the state. By incorporating historical weather patterns in to the knowledge base there may be a way to weight weather events by region or by county.
In conclusion, it has been shown that preventive IPM can be implemented through whole-farm planning. More specifically, crop-specific decision supports systems help facilitate preventive IPM.

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RECOGNITION OF TWO BIOCONTROL AGENTS AND THEIR LABORATORY EVALUATION FOR CONTROLLING WOOD BORERS

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Abstract

Recording of the natural enemies of wood-boring pests which attack date palm trees and apple fruit trees in Ismailia and Qalubeia governorates was explored. This aim was further supplied with the laboratory evaluation experiments of the gained biocontrol agents against their natural target borers. The present study revealed that two different types of natural enemies had been detected. These two natural enemies were the aphid lion, *Chrysoperla carnea* (Neuroptera: Chrysopidae) which was recorded attacking the leopard moth, *Zeuzera pyrina* (Lepidoptera: Cossidae) while the other one was the green muscardine fungus, *Metarrhizium anisopliae* (Hypocereales: Clavicipitaceae) which was recorded infecting the stages of red palm weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). Laboratory screening tests with 10^{11} spores/ml of *M. anisopliae* and the three larval instars of *C. carnea* were undertaken against RPW stages and *Z. pyrina* stages, respectively. Laboratory experiments confirmed the susceptibility of RPW stages to the infection with *M. anisopliae* spores resulting in an average mortality reached 63.4 and 45.9% for larval and adult stages, respectively. On the other hand the three larval instars of *C. carnea* showed considerable predation rates against *Z. pyrina* eggs resulting in 81.3, 87.4 and 47.3% mortality, respectively while this figure was 41.6, 47.8 and 38.9 % for the larval mortality, respectively. Obtained laboratory results showed the possibility for the usage of these two natural enemies to control the tested wood borers.

Keywords: *Records, Biocontrol agents, wood borers, Metarrhizium anisopliae, Chrysoperla carnea.*

Introduction

Leopard moth, *Zeuzera pyrina* (Lepidoptera: Cossidae) is an injurious wood borer attacking apple trees *Malus sp.* (Rosales: Rosaceae) causing severe economic damage. Also, Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is the most serious one which extensively attacks date palm trees, *Phoenix dactylifera* L. (Palmea: Palmaceae). Both apple and date palm trees are important economic crops worldwide. *Z. pyrina* and RPW are widely distributed boring pests infesting apple and palm orchards in many regions all over the world; in southern Asia, India, United Arab Emirates (UAE), Saudi Arabia, Iran, Egypt, Palestine and Jordan (Cox, 1993; Murphy and Briscoe, 1999 and Mani *et al.*, 2014).

In Egypt, both *Z. pyrina* and RPW stages are considered as major destructive and devastating pests causing a great significant damage as their stages existed around the year seasons and all over almost Egyptian governorates (El-Sebaey, 2004 and Merghem and Bibars, 2014). No method for their control is used except chemical control. Attempts to control these pests with chemical insecticides got limited success associated with development of insecticide resistance and pesticide residuals in nature. Extensive use of chemical insecticides resulted in environmental and economic problems such as hazard effects against human, beneficial animals and insects, and environmental pollution (Abo El-Saad *et al.*, 2001). Thus, alternative tools for effective, safe, and cheap control were encouraged to solve these problems. Among these tools was the use of the biological control measures either by their application

individually or by their use in integrated pest management (IPM) programs (Denny, 2005 and Roy *et al.*, 2006). Entomopathogenic fungi and natural predators are considered potential and environmentally safe biocontrol agents which are widely used to control many insect pests (Zimmermann, 1993). The green muscardine fungus, *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) and the aphid lion, *Chrysoperla carnea* (Neuroptera: Chrysopidae) are effective biocontrol agents against many insect pests (Driver *et al.*, 2000).
The present study aimed to explore and recognize the presence of the natural enemies of wood-boring pests attacking date palm trees and apple trees in Ismailia and Qalubeia governorates. This aim was further supplied with the laboratory evaluation experiments of the gained biocontrol agents against their natural target borers.

Materials and Methods

1. Survey, sampling and identification of the biocontrol agents of target wood borers:
Diseased RPW stages were examined and collected from infested date palm orchards in Ismailia governorate till spring of 2015. The stages were collected from trees with no previous insecticidal treatments. On the other hand, different developmental stages of the existed neuropteran species were also sampled from apple trees orchards in Qalubeia governorate to the laboratory for identification. The fungal culture was prepared using the naturally infected stages of RPW in Ismailia governorate and was identified on the base of the morphological characteristics of fungi as described by Barnett and Hunter (1999). Fungal isolate was confirmed by verification with postulates of Goettel and Inglis (1997). On the other hand, collected samples of the predator stages were examined for identification at Aphid-lion Rearing Laboratory in Plant Protection Research Institute, Dokki, Giza.

2. Collection of the target boring pests:
Healthy RPW and leopard moth stages were collected from infested date palm and apple orchards at the surveyed governorates. These collected stages were used for further experimental laboratory tests.

3. Laboratory screening tests:
Identified fungal isolate, *M. anisopliae* was tested against RPW stages collected from infested date palm trees orchards in Ismailia governorate. *C. carnea* larval instars were also released against the cossid borer stages sampled from Qalubeia governorate.

4. Propagation and screening of *Metarhizium anisopliae*:
The fungal isolate, *M. anisopliae* was used as a concentrated stock suspension to prepare 10^{11} spores/ml. concentration for preliminary screening and bioassay studies as described by (Yeo *et al.*, 2003).

5. Laboratory screening tests of the two biocontrol agents:
To test the efficacy of *M. anisopliae* isolate against RPW stages, five individuals of full grown larva or adult were used and exposed to 10^{11} spores/ml through topical application. In a second series of experiments, a check treatment of five individuals of each developmental stage was inoculated with distilled water as control. The glass jars were incubated for 15 days after treatment then mortality readings were recorded. Laboratory tests were also extended to test the predation of *C. carnea* instars upon *Z. pyrina* immature stages; eggs and larvae. Ten individuals of each larval instar of *C. carnea* were separately released to each borer stage. Five replicates for each treatment were made and mortality readings were recorded and corrected. The mortality percentages were recorded then corrected with Abbott's formula according to Abbott (1925).

Results and Discussion

1. Identification of the gained biocontrol agents:

Identification tests for the diseased RPW stages indicated the presence of the fungal disease namely *Metarhizium anisopliae* (Hypocereales: Clavicipitaceae). Plates for this fungus are presented in Fig. (1). Meanwhile, sampled stages of the neuropteran predator were mainly identified as *Chrysoperla carnea* (Neuroptera: Chrysopidae). Developmental stages of this chrysopid predator are shown in Fig. (2).

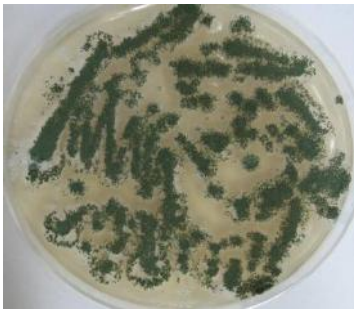
A.  B. 

Fig. (1): Plates (A&B) show the green muscardine fungus, *Metarhizium anisopliae*

A.  B. 

Fig. (2): Developmental stages of the aphid-lion, *Chrysoperla carnea*. (A. adult & B. immature larva)

Tables (1-2) showed the taxonomic positions of the two recorded natural enemies that infesting the host wood borers additionally to their target stages in relation to the existed locations and orchards either of apple and date palm trees in Ismailia and Qalubeia.

Table (1): Natural enemies attacking wood borers at apple and date palm orchards in Ismailia and Qalubeia governorates.

Scientific name	Common name	Systematic position	
		Order	Family
1- *Chrysoperla carnea*	Aphid-Lion Predator	Neuroptera	Chrysopidae
2- *Metarrhizium anisopliae*	Green Muscardine Fungus	Hypocereales	Clavicipitaceae

Table (2): Natural enemies and their host borers' stages in relation to their host plant trees in Ismailia and Qalubeia governorates.

Biocontrol agent	Borer species	Target stage	Host plant	Locality
C. carnea	*Z. pyrina*	Larval	Apple	Qalubeia
		Egg		
M. anisopliae	*R. ferrugineus*	Adult	Date Palm	Ismailia
		Larval		

Results obtained in Tables 1&2 elucidate *C. carnea* larval instars are natural enemies of the leopard moth as they were found to be predators for both eggs and larvae of *Z. pyrina* on apple trees at Qalubeia governorate. This finding is matching with Öztürk *et al.* (2005) in Turkey as they stated that the predator *C. carnea* was used on a narrow scale for the control of the cossid borer, *Z. pyrina*. Similarly, the gained results revealed that *M. anisopliae* fungus was noticed to be a natural enemy infecting the larval and adult stages of RPW that attack date palm trees at Ismailia governorate. This observation agrees with the results of Gindin *et al.* (2006) which confirms the infection of RPW with the fungus *M. anisopliae*.

2. Laboratory screening tests:

Results of the screening experiments with suspension of *M. anisopliae* at 10^{11} spores/ml concentration on stages of *R. ferrugineus* and those with the release of *C. carnea* larval instars against *Z. pyrina* stages are shown in Figs. 3&4. Obtained data revealed that these two biocontrol agents induced considerable mortalities for both those borers' stages.

The screening tests revealed that larval and adult stages of RPW are both susceptible to the infection with *M. anisopliae* fungus as shown in Fig. (3). The larvae showed a higher susceptibility to the fungal infection than adults as 63.4 and 45.9% mortalities were recorded for RPW larvae and adults, respectively. These obtained results were in accordance with Gindin *et al.* (2006) as they found that *M. anisopliae* were virulent against RPW under laboratory testing.

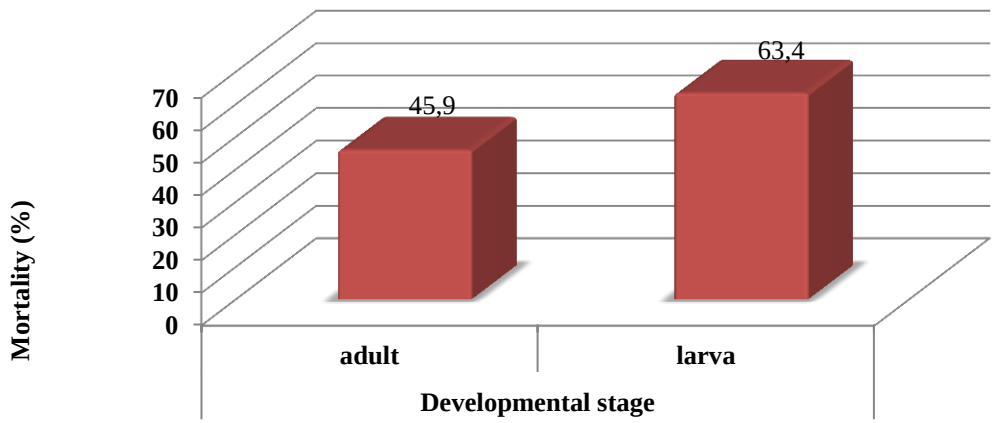


Fig. (3): Average mortality rate of *R. ferrugineus* stages caused by treatment with 10^{11} spores/ml *M. anisopliae* in the laboratory

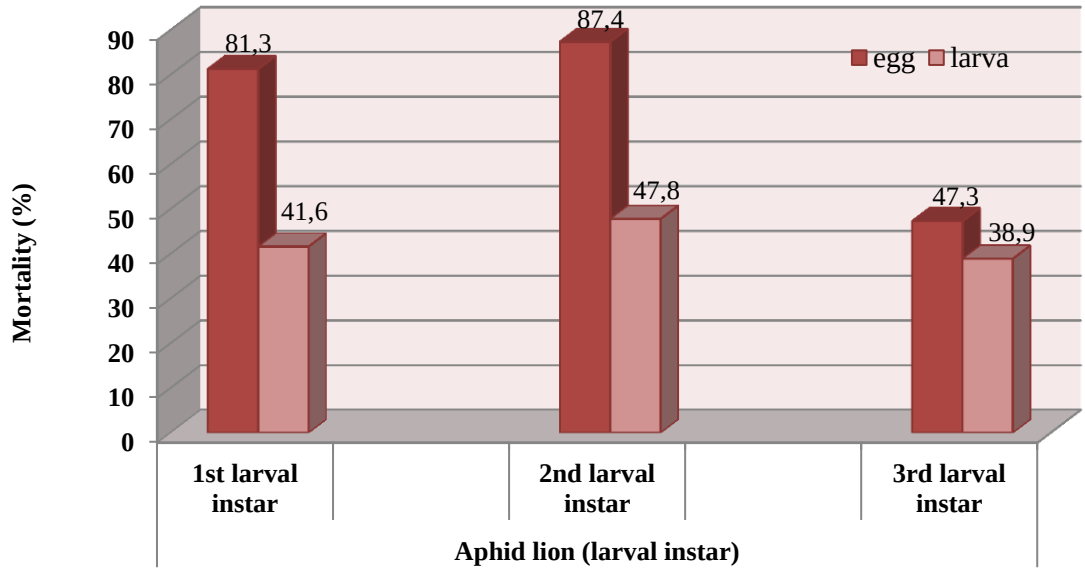


Fig. (4): Predation percentages of *c. carnea* larval instars against *Z. pyrina* stages

On the other hand, releases of aphid-lion larvae against immature stages of leopard moth resulted in high mortality levels for *Z. pyrina* stages. The 2nd larval instar of *C. carnea* revealed the highest predation rates against this borer stages recording 87.4 and 47.8% mortalities for its eggs and larvae, respectively.

Conclusion

Obtained results confirmed the presence of the two biocontrol agents*; C. carnea and M. anisopliae* naturally attacking *Z. pyrina* and *R. ferrugineus*, respectively. Furthermore, laboratory screening tests showed the possibility to use these biocontrol agents to reduce the infestation levels with those borers encouraging further field trials to assess the ability of these bio-agents to control both *Z. pyrina* and *R. ferrugineus* as host target borers.

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SURVEY ON DISEASES AND PESTS ATTACK QUINOA IN EGYPT

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Abstract

Quinoa, was introduced to Egypt through the few past years. According to available-data, no complete survey on pests and diseases attack quinoa in Egypt is available. The present work was designed to detect and identify diseases, and insects attack quinoa in five different Egyptian governorates (Giza, Fayium, Ismailia, Beheira and Monufia). Detection was carried out by examine different location, every 15 day and determine occurrence of diseases or pests. Data obtained showed that in all previously mentioned governorates, *Rhizoctonia solani* and *Macrophomena phaseolina* appeared as aggressive causal organism for root rot and damping off disease for young quinoa seedling. *Fusarium solani* was isolated only from samples were collected from Giza location. *Peronospora farinosa* f.sp. *chenopodii* was detected in all locations. This disease appeared during vegetative growth stage and before flowering or just after flowering. Insects survey was also carried out. Attacked plants were collected and transferred in ice box to laboratory for further identification. Data obtained showed that two aphid species (*Myzus persicae* and *Aphis gossypii*, Hemiptera) were detected in all location. Two pests belong also to order Hemiptera were detected in Ismailia governorate during vegetative growth and flowering stages. These two pests were identified as (*Nysius cymoids*) whereas the other was (*Creontiades pallidus*). Another Hemiptera pest, Cotton mealy bug (*Phenacoccus solenopsis*) was detected only in Giza on individual plants at seed formation stage. Only, in Faiyum governorate, a weevil belongs to Coleoptera (*Sitophilus granaries*) was detected during late flowering and grain formation stages (April- May), on few individual plants. A shoots feeder belongs to Diptera, *Atherigona theodori* was found in Ismailia and Faiyum. This pest penetrate plant stem and feed on inner stem content eventually causing wilt and collapse for infected plants. This pest was recorded on few individual plants. *Tuta absoluta* (Lepidoptera) was also detected in Giza, Faiyum and Ismailia. It attacks fresh green leaves. Cotton leaf worm (*Spodoptera exigua*) was detected in Faiyum on very few plants during early stages of growth (October).

Keywords: *Quinoa – Egypt – pest – disease*

Introduction

Quinoa (*Chenopodium quinoa* Willd., Caryophyllales, Amaranthaceae) is considered as a very important grain crop for the Andean region of Latin America. It was domesticated 3000 – 4000 years ago for human consumption (Shukla and Ohri, 2006). Quinoa has a high nutritive value because its grains contain high protein quality and quantity as well, all essential amino acids and trace elements and vitamins. Quinoa has the ability to adapt to different ecological environments and climates (Rasmussen *et al*., 2003 ; El- Assiuty *et al*., 2014). Plants can be grown under poor, saline soils conditions. For all these advantages quinoa can be considered as the most promising crop especially in poor new reclaimed rural area. According to (Danielsen *et al*., 2003) quinoa is more susceptible to soil borne pathogens in comparison with other species belong to the same family. Quinoa is attacked by several pests and diseases in many south American countries (from Colombia in the north through Ecuador, Peru and Bolivia to Chile in the south). These pests and diseases attack all plant parts during all stages of growth (Drimalkova and Veverka, 2004).

The aim of the present study is to survey pest and disease that may attack quinoa in different Egyptian governorates. These studies will offer a map and data regarding distribution of disease and pest attack quinoa. High authority and decision maker can use these data to build up their decision or plans on scientific base and also prepare the suitable mean to control each pest and disease.

Materials and Methods

Commercial Quinoa seeds were used in this study. Quinoa seeds were planted in five different governorates, to study disease and pest that can attack this crop at different climatic conditions, different type of soil, and at different degrees of salinity (280 – 1800 ppm) in irrigation water. These five governorates were (Giza, Fayium, Ismailia, Beheira and Monufia).

Quinoa seeds were sowed during last week of September in rows 70 cm apart. Five to ten seeds were planted in holes distanced at 25 cm. All plants received fertilizers at the rate of 90: 40: 100 N: P: K in addition to two sprays with calcium chloride and two sprays with trace elements (Fe – Zn –Mn and Mo). Different locations were examined every 15 day to detect diseases and pests presence. Suspicious samples that showed presence of disease symptoms or insects attack were collected and transferred to laboratory for further analysis. All pathogenic fungi were identified at the central laboratory of organic agriculture. Isolated, purified fungi or fresh plant materials with symptoms of downy mildew were used to identify different pathogens according to their morphological characteristics .*Rhizoctonia solani* was identified according to key developed by **Sneh *et al*. (1992);** *Fusarium* spp. according to **Booth (1971)**; *Macrophomina phaseolina* according to **Ellis (1976)**; *Peronospora farinosa* was identified according to **Choi *et al*. (2010)**. Samples of quinoa attacked by insects were transferred in ice boxes to laboratory. All plants attacked with registered larval presence were kept in separate jars with pieces of quinoa plants under room temperature to allow larvae to complete life cycle in laboratory conditions. Larvae, pupae and adults were sent to the museum of Entomology Department, Faculty of Science, Ain Shams University for identification.

Results and Discussion

The selected five governorates were chosen to represent different type of soils, water salinity and weather conditions. In Giza, soil belongs to clay type with high water holding capacity, flood irrigation system with Nile water (280 – 350 ppm), while the weather is almost dry warm. Faiyum is located about 100 km to south west from Giza with light clay soil and moderate water holding capacity. Salinity of irrigation water is (about 350 ppm). Beheira governorate about 230 km northern from Giza. The selected farm in this governorate has sandy soil with low degree of water holding capacity. Drip irrigation system with slight saline water (700-1000 ppm), wet cold weather. The governorate Monufia is located in the delta of Nile river about 120 km north of Giza. The soil is clay with high water holding capacity the weather is wet and cold, flood irrigation using Nile water (280-350 ppm).The governorate Ismailia is located about 130 km eastern from Giza with sandy soil, low water holding capacity, drip irrigation system, saline water (1200–1800ppm) and warm wet weather. Samples were collected periodically every 15 days. Quinoa plants were examined visually using binocular or light microscope to identify the causal organism. Pure samples from isolated pathogens or fresh leaves show identical symptoms (for obligate pathogen) were examined and systematically identified according to approved identification keys. Data obtained in this survey are illustrated in Table (1), Table (2) and Fig1.

Data in Table (1) show that *R. solani* and *M. phaseolina* appeared as a very aggressive pathogens and cause damping off on new sprout. To avoid these diseases quinoa seeds must

be treated with seed disinfectant prior sawing in soil. This disinfectant may be biocide (**Abd El-Moity, 1985**) or chemical preparation (**Anonymous, 2011**). Downey mildew also appeared as a sever disease for quinoa. Copper preparation can be used before flowering stage to protect quinoa against this disease (**Chaudhry *et al.*, 2009**).

Data in Table (2) indicate that Aphids, *Tuta absoluta* and cotton leaf worm can attack quinoa causing considerable loses. These pests can be monitoring by adhesive yellow sheets or pheromone traps and controlled with suitable means either chemical or biological means according to (Approved recommendation for agriculture pest control, **Anonymous, 2011**).

Some pests appeared only on very few individual plants. Such as, shoots feeder and cotton mealy bug. These insects must be monitoring using suitable traps , avoid repeating quinoa or wheat cultivation in the same field for two successive years to reduce pest problem and to prevent spread of these pests.

Table 1: Pathogens detected on quinoa in Egypt.

pathogens	**Location / period of occurrence**	**Host range**	**Infected parts of the host plant and type of damage**
Soil borne pathogens			
Ceratobasidiales Ceratobasidiaceae) *Thanatephorus cucumeris* (teleomorph) *Rhizoctonia solani* (anamorph)	Giza, Fayium, Beheira, Ismailia Monufia/ Seedling stage Wide spread with serious damage	*R. solani* causes root rot and damping –off for quinoa and many commercially cultivated crops and causes significant damage for seeds and yield.	Young sprout are attacked followed by pre-emergence damping off or mature seedling are attacked with post-emergence damping off. In old plant, brown lesion on the main root and discoloration in hair roots are detected leading to reduction in yield (Manici *et al*., 2005).
pathogens	**Location / period of occurrence**	**Host range**	**Infected parts of the host plant and type of damage**

Botryosphaeriales Botryosphaeriaceae *Macrophomena phaseolina*	Giza, Fayium, Beheira, Ismailia Monufia/ Seedling stage Wide spread with serious damage	*M. phaseolina* attacks many hosts include: peanut, Cabbage, pepper, chick pea, strawberry, soybean, sunflower, sesame, potato, sorghum, corn. and quinoa as detected	Attacks roots and causes charcoal rot. Reducing root system capacity consequently reduce yield. (Zveibil and Freeman, 2005)
Hypocreales **nectriaceae** *Fusarium solani*	Giza Seedling stage In few plants	*F. solani* cause root rot for many hosts include potato, bean, cucurbits and quinoa as detected.	Attacks all plant parts in the soil. At early stage causes damping off, while in older plants discoloration and root rot. This attack reduces yield quality and quantity. (Booth,1971)
Downy mildew			
Peronosporales Peronosporaceae *Peronospora farinosa*	Giza, Fayium, Ismailia, Beheira, Monufia/ before flowering Wide spread with serious damage	Obligate parasite, on many Chenopodiaceous family host plants, (ex: sugar beet, quinoa and spinach).	Attack plants through stomata, mainly lower surfaces, it can severely reduce the growth of plants and yield under favorable conditions. (Choi *et al*., 2010)

Table 2: Insects detected on quinoa in Egypt.

No	Insects	Location / period of occurrence	Host range	Infested part of the host plant and type of damage
1-	Hemiptera Miridae *Creontiades pallidus* (green mired)	Ismailia Blooming stage	Cotton, Clover, Alfalfa, Corn, Grasses and sorghum. (Buyckx 1962)	It damages many types of field crops. Nymphs and adults both feed on young bolls or head of sorghum or on quinoa as detected in this work. (Wheeler 2001)
No	**Insects**	**Location / period of**	**Host range**	**Infested part of the host plant and type of damage**

		occurrence		
2-	Hemiptera Lygaeidae *Nysius cymoides*	Ismailia / Blooming stage	Cruciferous crops, Jojoba, Canola, Tobacco, Grape. (Gentry 1965)	It causes damaging to seed pods of crucifers or head of quinoa as detected. (Beardsley 1971, Parenzan 1985)
3-	Hemiptera Sternorrhyncha *Pseudococcidae –Phenacoccus solenopsis* (cotton mealy bug)	Giza/ Harvest time	This bug attacks numerous crops, weeds, ornamentals and medicinal plants. Cotton is one of its most favored hosts, (Ibrahim *et al.* 2015)	Larvae leaves of the plant turns yellow and becoming crinkled or malformed. It infests the leaves, fruit, branches, main stems, feeding on phloem sap and egesting copious, sugary honeydew. (Dutt 2007, Hodgson *et al.* 2008)
4-	Hemiptera Aphididae *Aphis gossypii* *Myzus persicae-*	Giza, Fayium, Beheira, Ismailia Monufia/ All growth stage	polyphagous (Abou-Elhagag 1998, Capinera 2001) -highly polyphagous (Blackman and Eastop 1984).	Aphids suck nutrients from the plant. Their feeding causes a great deal of distortion and leaf curling, hindering photosynthetic capacity of the plant. (Abou- Elhagag 1998, Margaritopoulos *et al*. 2006) It can attain very high densities on young plant tissue, causing water stress, and reduced growth rate of the plant. It can cause appreciable reduction in yield of root crops and foliage crop. (van Emden *et al*., 1969)
5- **No**	Coleoptera Curculionidae Dryophthorinae *Sitophilus granaries* (wheat weevil or grain weevil) **Insects**	Fayium/ Seed formation **Location / period of occurrence**	Wheat, Barley, Maize, Sorghum, Oats, Rice(Alfieri 1976, Trematerra *et al.* 1999), and quinoa as detected. **Host range**	Weevils (Larva as well as the adult) causing destruction of grains and reduce yield quality and quantity. The developmental stages of *S. granarius* are not normally seen as they occur inside intact grains. Adult emergence holes with irregular edges are apparent some weeks after initial **Infested part of the host plant and type of damage**

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				attack. Adults can be found wandering over the surface of grain especially if the grain has been disturbed. (Longstaff 1981)
6-	Diptera Muscidae *Atherigona theodori*	Ismailia, Fayium/ Harvest time	Sorghum, Maize, Wheat, Rice, Sugarcane, Alfalfa. (Deeming 1971, Reddy e*t al.* 1981, Pont and Deeming 2001), and quinoa as detected	The larvae cut the growing point, resulting in wilting and drying of the infected plants and produce a bad smell. (Pont and Magpayo, 1995)
7-	Lepidoptera Gelechiidae *Tuta absoluta*	Giza, Fayium, Ismailia/ All growth stage	Tomato, Potato, Eggplant, Tobacco. (El-Arnaouty and Kortam, 2012), and quinoa as detected	The larva feeds voraciously upon tomato plants, producing large galleries in leaves, burrowing in stalks, and consuming apical buds and green and ripe fruits. It is capable of causing a yield loss of 100%. (Desneux 2010)
8-	Lepidoptera Noctuidae *Spodoptera exigua*	Fayium / seedling stage	The wide host range of beans, corn, peas, sugar beets, celery, lettuce, potato, tomato, cotton, cereals, oilseeds and many flowers. (Wilkerson *et al.* 2005, Robinson *et al.* 2010), quinoa also consider as a host as detected.	The larvae feed on the foliage of plants, and can completely defoliate small ones. Smaller larvae devour the parenchyma of leaves, so that all that remains is the thin epidermis and veins. Larger larvae tend to burrow holes through thick areas of plants. (Tingle and Mitchell 1977)

Conclusion

According to results obtained from this study it can be concluded that quinoa plants can be successfully cultivated with considerable yield at different degrees of water salinity till 1800 ppm. It can also be grown under wet or dry weather and in sandy or clay soil. Variation in yield due to these factors needs another study to exactly determine variation in yield. Our results also showed that quinoa is attacked by numerous pests and diseases. The most destructive disease is root rot and damping off caused by *R. solani* and *M. phaseolina.* Data also show that quinoa plants are susceptible to downy mildew. Aphids, *Tuta absoluta* and cotton leaf worm can attack quinoa plants and cause considerable damage for yield. Some

other pests were appeared only on few individuals plants such as shoots feeder and Cotton mealy bug. Special studies will carry out to exactly determine losses can be occurred due to these diseases and pests.

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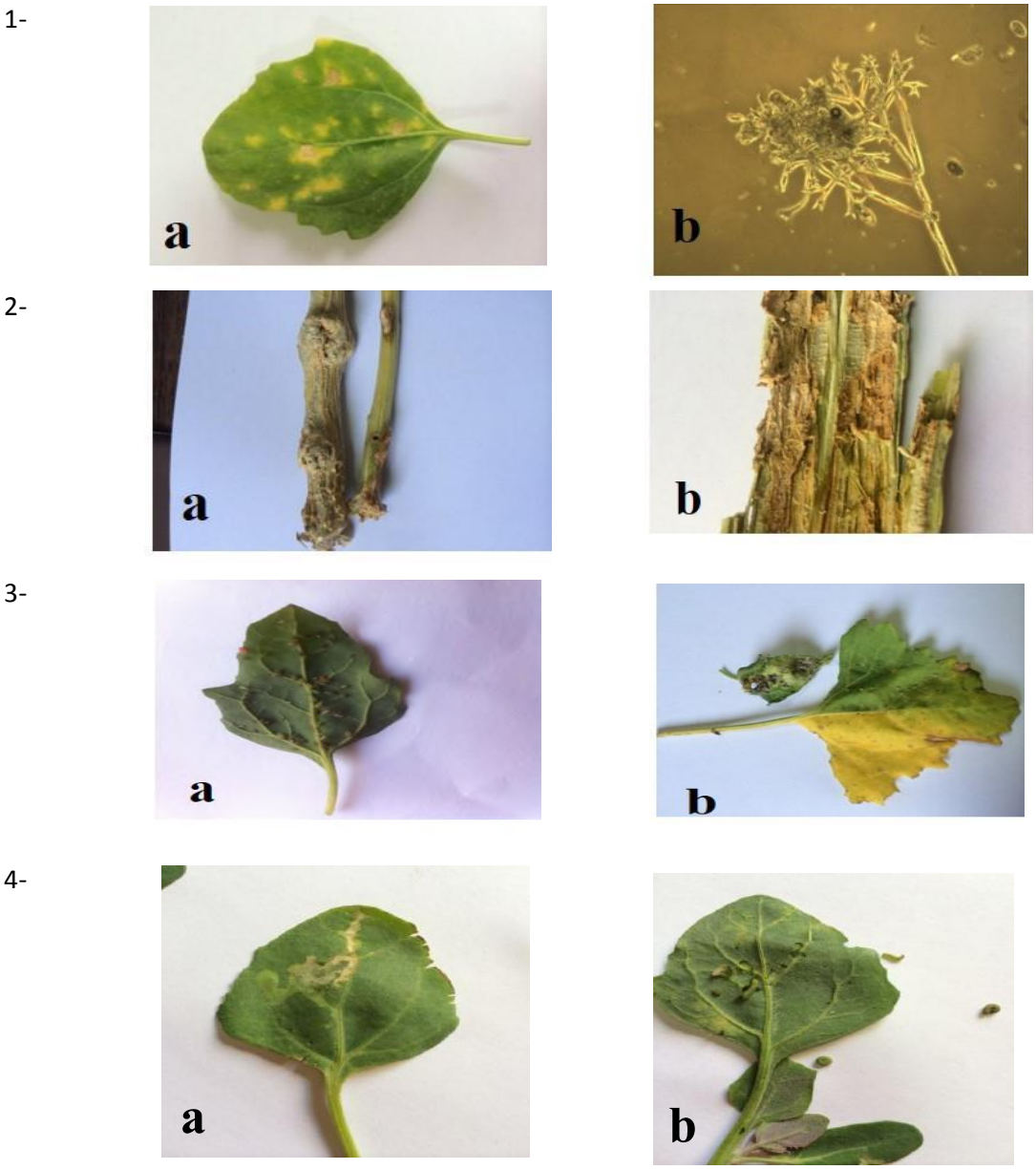

Fig 1. Symptoms and causal organisms of pests and diseases attack quinoa

1-Downy mildew a) Symptoms b) Pathogen

2- Shoot feeder a) Damage on plant b) Larvae

3- Aphid a) Symptoms b) Damage

4- a) Damage due to *Tuta absuluta.* b) Damage and larvae of cotton leaf worm

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AN INNOVATIVE MACHINE PERFORMING THE FLAME TREATMENT OF KIWI FRUIT PRUNING RESIDUES INFECTED BY PSA

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Abstract

Bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* (PSA) is affecting kiwifruit cultivation worldwide. In infected orchards the removal of diseased vegetation is essential for control. The bacterium can survive on woody residues becoming a source of inoculum for subsequent infections. Open burning of prunings is a common orchard management practice, shredding of residues is a feasible alternative but disease issues in kiwifruit would require actions to disinfect them first. In this context an innovative flame treatment prototype was recently developed for processing the prunings directly in the aisle. The tractor drawn prototype has two main parts: a shredding unit and a heating unit. The horizontal flail blade rotor shreds the prunings, the thin layer of wood chips flow into the heating unit, where LPG burners produce a turbulent flux of hot gasses increasing the temperature of residues for sterilization. Preliminary field tests were conducted to assess treatment effectiveness in relation to thermal dose, treatment speed and mass of residues per unit area. Forward speeds compared were 700 and 1400 m/h, resulting in an exposure time to the flame of 4 and 2 seconds, respectively. Treatment effectiveness was assessed through biological assays on treated and untreated samples taken from infected vines. Flame treatment disinfected the treated samples, but the bacterium was isolated from the untreated samples and PSA identity was verified via PCR analysis. Hourly working capacity and fuel consumption were estimated for both forward speeds. The machine is especially intended for organic farming systems.

Keywords: *flame, LPG, disinfection, prunings, PSA bacterial canker*

Introduction

Bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* (PSA) has spread to all major kiwifruit growing regions and is endangering kiwifruit crops worldwide (Vanneste et al., 2011). Since 2008, the emergence of a severe epidemic in Italy was caused by a highly virulent strain. (Ferrante et al., 2010). The outbreak has caused important economic loss in Italy and a decline in cultivated surfaces, after the removal of devastated orchards and a stop in new plantings.

Control of bacterial canker has required the adoption of extraordinary protection measures, causing an increase in production costs. All cultivated varieties (*Actinidia deliciosa* and *A. chinensis*) are affected by PSA and curative treatments for bacterial canker do not exist (EPPO, 2012). Conventional control strategies, based on use of existing chemicals, are successful to some extent against PSA. Phytotoxicity, resistance development and regulatory measures restrict the use of copper compounds and antibiotics, which are the key chemicals used to fight bacterial plant diseases. (Cameron et al., 2014).

Synergy between different crop protection strategies is necessary. Additional phytosanitary measures for prevention and containment are essential to fight the spread of PSA. Prevention requires continuous inspection of the fields for early detection of symptoms and early diagnosis of diseased plants. Containment is achieved by eliminating infected plants or plant

parts to reduce field inoculum. The timely removal of prunings from the field can also be effective for sanitization purposes, since it has been proven that PSA can survive on residues for some time (up to 45 days), and therefore those left lying on the orchard floor represent a further source of inoculum for subsequent infections in the orchard (Scortichini, 2012). Kiwifruit plants have a very exuberant vegetative growth, therefore winter vine pruning drops a great amount of wood residues, up to 10 t ha^{-1} (fresh weight) on the orchard floor (Nati et al., 2011). Infected fields benefit from more intensive pruning to remove older vines (2 to 4 years of age), in these plant structures the bacterium lives endophitically. Reducing the amount of permanent wood improves disease containment (Mazzaglia et al., 2011). Summer pruning is carried out on a monthly basis to shorten new shoots and replacement canes, starting a couple of weeks before blossom time. Herbaceous residues from infected plants may also be a source of inoculum. The open burning of prunings is a common practice in orchard management for disposing of residues and burning is nowadays the main destination of kiwifruit prunings. The application of fire, also referred to as 'thermal sanitation', is helpful in reducing the incidence of plant pathogens (Desilets, 1997). Burning of overwintering inoculum on fallen leaves reduces apple scab (*Venturia inaequalis*). Control of dieback (*Diaporthe vaccinii*) and canker (*Godronia cassandrae*) in blueberries was tested through burning of crop residues (Hardison, 1976). Open burning at the orchard site doesn't require a specific mechanization, for this reason it is often preferred as an effective solution, although handling of residues requires high working time and high costs. Furthermore some twigs escape collection and remain on the orchard floor, carrying inoculum. There are additional options for handling prunings that are intended for biomass valorization. For example kiwifruit prunings are often shredded directly in the field, leaving the residues in place to return organic matter to soil. The recovery of biomass for use in energy generation or for composting is another alternative, adding value to residues. Although shredding is technologically feasible, when cleaning up the orchard floor in infected kiwifruit orchards disease issues are a concern and the phytosanitary legislation in force prohibits mulching and transportation of infected material to other sites. Farmers must therefore consider the adoption of additional measures to disinfect the residues before shredding, in order to avoid the spread of inoculum in the field. In this context, 'OFFICINE MINGOZZI', a company specialized in the construction of flame weeding equipment and machines for horticulture, has developed an innovative technology to process kiwifruit prunings directly in the aisle. (Tomasone et al., 2014). The heat treatment is intended to reduce field inoculum, so that the shredded material can be safely released on the ground. The same technique is also proposed for removing the inoculum carried on fallen leaves and residues produced after summer pruning operations.

The aim of the present paper has been to determine the effectiveness of the flame technique in achieving a reduction of the inoculum, performing the treatment on naturally infected vines, pruned from diseased plants and treated by shredding and heating in a field trial. The experiments also were intended to establish the choice of operating parameters in field treatments, in relation to the amount of residue to treat, to the speed of the tractor assembly and to the arrangement of materials on the ground, determining the dose of gas per surface unit in the orchard.

Materials and methods

The machine was designed to perform two operations in a single-step, i.e. to shred all the residues and simultaneously to heat them via LPG flaming. By design, the tractor-mounted prototype was limited in size and weight, and made suitable for use with compact power class tractors, the most common in orchards. The two main components in the assembly are the shredding unit and the heating unit (Figure 1). The tractor, moving along the row, picks up the pruning residues, which are immediately shredded by the front flail rotor, producing a thin

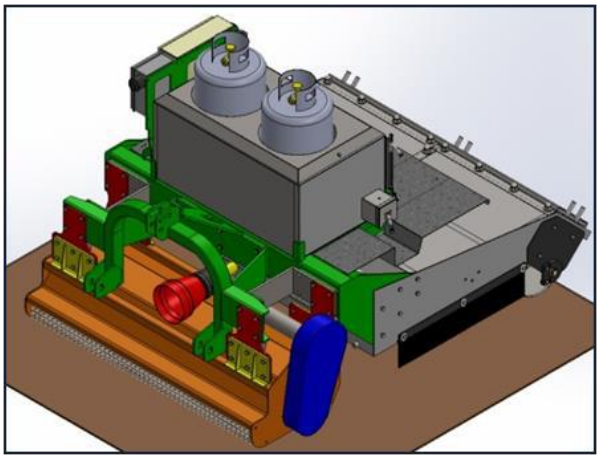

Figure 1: CAD images of the combined assembly

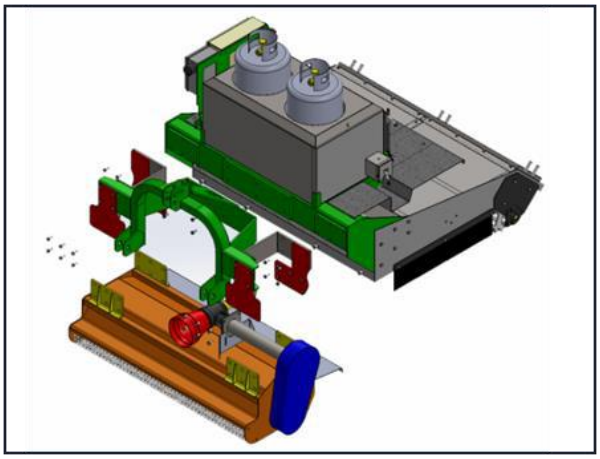

Figure 2: CAD schematic of the bolted linkage

layer of wood cuttings delivered rearward into the heating unit, amongst the open flames. The horizontal flail rotor, perpendicular to the row, carries a series of standard "Y" shaped flail knives, attached to the rotating shaft by means of brackets. In total 56 flail blades are arranged on 8 staggered rows, providing an effective cut. The PTO drive is set at standard 540 rpm speed. Front and rear rollers are included to maintain the correct work height as the machine moves over the ground. The unit is provided with a structural frame for attachment to the tractor's three-point hitch and for the connection with the heating unit placed behind. Dimensions of the shredding apparatus are 1500 x 960 x 855 mm (Length x Width x Height) weighing 260 kg, cutting width is 1400 mm and range of power requirement is 26-30 kW.

The heating unit is connected to the flail shredder by means of bolted metal plates. The two units are easily disconnected for separate use, either mowing and shredding or broadcast surface flaming, respectively (Figure 2). Two LPG steel cylinders (25 kg capacity each) are carried in a tank filled with warm water: the water bath supplies the thermal energy required as latent heat for the vaporization of LPG from liquid to gas, when gaseous LPG is drawn at high flow rates, maintaining constant pressure and constant flow rate to the burner's nozzle.

The heart of the unit is the hot chamber: it is closed above by an insulated stainless steel hood, it is connected in the front to the flail shredder, on each side metal chain curtains close the gap between frame and soil, in the rear a cage roller acts as the chimney to extract the hot exhaust fumes. The flames are fired by an array of 8 burners, aligned across in a single row, evenly spaced from side to side (Figure 3).

Figure 3: intensive flames under the heat chamber

Figure 4: field treatment of prunings along the row

The gaseous fuel burner used on the machine is of the premixed type, in which the gas and air are mixed before passing through the nozzle. The stability of fuel combustion was checked, reducing the negative effect of the air turbulence produced by the rotating flails and by the mass of cuttings flowing into the combustion chamber. The burners were studied to give a stable flame, burning gas efficiently and producing a powerful turbulent flux of hot gasses, maintaining a high temperature inside the chamber, constantly above 650 °C.

The operative parameters used for the field trials were as indicated: gas flow rate was set at 3.0 kg h^{-1} per torch at an average gas pressure of 0.14 MPa, for a nominal thermal power of about 38 kW per burner; the resulting total hourly gas consumption is about 24.0 kg h^{-1}, for an overall power of about 304 kW. The flaming unit is operated by the driver directly from the tractor's cabin by means of an electronic connection box having a magnetic base, placed in the cabin for remote operation.

Field trials

Field tests were conducted with the innovative prototype in order to assess bactericidal effectiveness of the treatments carried out on naturally infected pruning material, in relation to the thermal dose and to flame exposure time. The prunings were collected in an infected kiwifruit orchard and were arranged in windrows at the centre of the aisle (4.5 m row spacing) in order to complete the treatment through a single pass along the row (Figure 4). The symptoms of the disease were assessed visually on some vines taken from infected trees. Symptoms were identified on the vines as red spots beneath the lenticels, also brown streaks in the subcortical tissues when the bark is peeled from the infected vine (Figure 5). These infected vines were marked with spray-paint and placed in the windrows along with the other prunings. Following the heat treatment the vines are shredded and samples of these coloured cuttings were collected to be used for the microbiological analysis in the laboratory. Prior to the treatment, control samples were also taken from these coloured vines to be used as the untreated control, for the comparison of the bacterial load carried by the residues before and after the heat treatment.

The prunings were treated with the innovative machine making a single pass over each row, simultaneously shredding and heating the prunings for disinfection. The thermal power employed in field operations was fixed by setting the gas pressure gauge at a constant 0.14 MPa. The thermal dose is thus derived as a function of both treatment speed and mass of residues per unit area. Two forward speeds were compared in the tests: a slow 750 m/h forward speed and a fast 1500 m/h, the latter being a drive speed that is common for ordinary vine shredding operations. The amount of pruning residues present on the orchard floor was measured by sampling segments of known length along the windrow and weighing the mass of the sample. The time required by the heat chamber to move over the material deposited on the ground is easily calculated for each of the two speeds. The heat chamber is 1.25 m in length and so the material on the ground is under the effect of the flames for a time of exposure corresponding to 6 and 3 seconds, for the slow and fast work speed, respectively (Figure 6).

These two plots have been referred to as the long and the short treatment time. Microbial assays were carried out to verify the effectiveness of the disinfection of the treated samples, compared to the control samples (not treated) taken ahead of treatment from the same infected vines. In the laboratory, using aseptic technique, portions of tissue were taken from the samples and put into a sterile saline solution, held in oscillation for 45 min so that all the microorganisms could move to the liquid medium. Next, aliquots (100 uL) of the solution were taken to be transferred into test tubes containing nutrient broth for liquid culture.

The tubes were incubated at 25 °C for 24 h and subsequently bacterial growth was assessed by analyzing the turbidity of the broth. Isolation of bacteria was later done in Petri dishes with solid substrate NSA, streaking tenfold dilutions for the isolation of colonies. The following

step for the identification of PSA was performed by molecular analysis, via duplex-PCR according to the diagnostic protocol described by Gallelli et al. (2011).

Figure 5: symptoms on vines, spots and streaks

Figure 6: heat chamber seen from underneath

Results and discussion

The microbial culture in liquid broth established the absence of microbial growth for all the samples collected from the flame treated residues, for both the 750 and the 1500 m/h treatment speeds. Instead the assays with the untreated control samples have seen the liquid broth turn opaque after 24h of incubation, meaning that PSA was indeed present on the symptomatic vines taken from the infected plants. The bacteria isolated from the control samples was identified as PSA bacterium by molecular analysis.

For the given amount of residues in the row, a good efficacy of disinfection was reached already with the slow 3 seconds treatment time. In the specific situation of the field trials conducted, the total mass of pruning residues present on the ground weighed on average 5.5 t ha^{-1} in fresh weight. This quantity is about half the amount of total residues that can be produced annually by pruning in winter a kiwifruit orchard in full production, having a ordinary tree density of about 550-620 plants per hectare. In the case of more intensive winter pruning and thus more residues on the ground, the same ratio of thermal dose per unit mass of residue can be achieved by reducing the speed of the treatment appropriately.

Table 1: Operative parameters in field trials

Plot	measure unit	slow speed plot	fast speed plot
treatment speed	(m h^{-1})	750	1500
heat exposure time	(s)	6	3
hourly work capacity	(ha h^{-1})	0.3375	0.6750
work time	(h^{-1} ha)	2.96	1.48
LPG consumption	(kg ha^{-1})	71	35
cost for LPG (current prices)	(€ ha^{-1})	124	62

The operative results achieved by the machinery in the tests conducted are shown in Table 1. The hourly working capacity and the consumption of LPG fuel are acceptable when treating at 1500 m/h. The slow speed tested might be necessary for greater quantities of residues to be reclaimed in the orchard, but in that case the feasibility of the treatment from an economic point of view needs to be further considered.

Conclusions

The field tests have shown so far very good results, but further tests are needed to define the parameters when treating different quantities of residues, for establishing the consumption of

LPG fuel and all the costs associated with the treatment. This machine may be used both in conventional and organic farming because the LPG is allowed by relative crop regulations. The heat treatment could give a hand towards reducing the chemical sprayings in the orchard. Following disinfection, the organic material provided by prunings could be safely shredded in place in the orchard, accumulating organic material on the orchard floor that will definitely contribute to the content of soil organic matter and to soil fertility, otherwise all the residues should be removed and burned outside of the orchard. The treatment could be proposed for a more general application regarding other fruit species, intended furthermore to achieve the remediation of the orchard site after winter pruning, disinfecting the residues.

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EFFECTS OF CARBON SOURCE ON THE TOXINOGENESIS OF PENICILLIUM AURANTIOGRISEUM

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Abstract

Currently, many pathogens are at the origin of disease and considerable economic losses. By secretion of highly toxic substances, the toxigenic fungi are a real danger to human and animal health.

The *Penicillium genus* is considered as especially useful fungi in the food and pharmaceutical industry. However, some studies have shown that they secrete certain mycotoxins. This toxicity may appear even for useful species, in cases when some physicochemical or trophic factors are changed. *Penicillium aurantiogriseum* (known by its lipase activity) is a species that frequently contaminates olives and other foodstuffs namely the cereals and derivatives. Because its mycotoxins that produces, *Penicillium aurantiogriseum* is used in food and pharmaceutical industry.

The strain used in this study was identified as *P. aurantiogriseum* based on morphological basis according to Pitt's key of determination. Also, its toxic profile was determined by thin layer chromatography.

The analysis of the metabolic profile revealed three mycotoxins in *P. aurantiogriseum* (terrestric acid, penicillic acid and aurantiamin). The nature and amount of thoses toxins were tightly related to the nature and concentration of carbon source in the artificial rearing medium of *P. aurantiogriseum*. The highest productivity of terrestric acid was obtained on Yeast Extract Sucrose broth and the production of this toxin increased with sucrose concentration in this medium. Penicillic acid was produced in different media (CYA, YES and MB) while the best medium for aurantiamin excretion was glucose-free Medium Base.

Keywords: *Penicillium aurantiogriseum; mycotoxins; thin layer chromatography; carbon sources.*

Introduction

Genus of Penicillium has long been used in food and pharmaceutical industry. However, some species are toxinogenic and, thus, could be very harmful to human health.

Numerous studies have reported that some mycotoxins can be secreted by a huge number of *Penicillium* species **(Lu et al. 1994)** including those used in food processing and pharmaceutical industry, whenever favorable physicochemical and trophic conditions are present.

Penicillium fungi contaminate food during storage. *P. aurantiogriseum* that is frequently isolated from food **(Borjesson,L, Stollman, and Schnurer, 1990; Khaddor et al. 2007; Maouni et al. 2001)** secrets three mycotoxins: terrestric acid, penicillic acid and aurantiamin. On the other hand, it was also found that aurantiamin has an antibacterial effect against *Bacillus subtilis* **(Khaddor et al. 2007)**. Moreover,due to its chemical structure which is similar to phenylahistinand registered activity against tumor cell lines, aurantiamin is considered as a potential substituent to colchicin **(Boulikas and Tsogas 2008)**.

The aim of this work is to study the effects of different culture media compositions on toxinogenesis of *P. aurantiogriseum*, particularly the influence of glucose and sucrose used at different concentrations.

Materials and methods

The *Penicillium* aurantiogriseum strain

The *P. aurantiogriseum* strain used in this study belongs to the collection of EFBRT (Environmental and Food Biotechnology Research Team).
The strain was first cultivated on MEA (Malt Extract Agar) at 25 ° C for 7 days, and the spores were suspended in 0,1 % of tween 80. The density of the suspension was adjusted to 10^7 spores / ml.
Purification was then made on three different media MEA, CYA (Czapeck Yeast Extract Agar) and G25N (Glycerol 25% Nitrate Agar) (table 1). The identification was realiseed according to the determination key of Pitt **(Pitt 1988)**.

Culture conditions

The media used for toxinegesis study were YES (Yeast Extract Sucrose), BM (Base Medium) and G25N. The composition of all media is given in Table 1.
For this study 25 ml of YES and MB liquid media, with different concentration in sucrose or glucose were distributed in 100 ml Erlenmeyer flasks and inoculated with 2ml of spore suspension. All cultures were incubated for 10 days at 25 °C.

Table 1. Media used in this study and the concentrations of glucose and sucrose tested

Basic medium			Carbon source	
Name	Composition	Reference	Nature	Quantities added (g/l)
Media of reactivation and purification of *Penicillium aurantiogriseum*				
CYA (Czapeck Yeast Extract Agar)	Czapeck concentré : (10ml) ; K_2HPO_4 : 1 g ; Yeast extract : 5g ; Sucrose : 30g ; Agar : 15 g ; Distilled water:1000ml.	(Pitt 1988)	Sucrose	
MEA (Malt Extract Agar)	Malt extract agar : 30 g Glucose : 20 g Agar : 10 g ; Distilled water:1000ml	(Pitt 1988)	Glucose	
G25N (Glycerol 25% Nitrate Agar)	Concentrated Czapeck* : 7.5 ml ; K2HPO4 : 0.75g ; Yeast Extract : 3.7g ; Glycerol : 250g ; Agar : 12g ; Distilled water : 750 ml	(Pitt 1988)	Glycerol	
Media inducing toxinogenesis of *Penicillium aurantiogriseum*				
YES (Yeast Extract Sucrose Broth)	Yeast Extract : 20g ; Sucrose : 100g ; Distilled water : 1l	(B. Botton 1990)	Sucrose	0; 100 ; 200 ; 300

BM (Base Medium)	Malt Extract : 130g ; Agar : 15g ; Distilled water : 1l	(Pitt 1988)	Glucose	0; 100 ; 200 ; 300
*Concentrated Czapeck: $NaNO_3$: 30g ; KCl : 5g ; $MgSO_4$:5g ; $FeSO_4$: 0,1g ; $ZnSO_4$: 0,1g ; $CuSO_4$: 0,05g ; Distilled water : 100 ml.				

Research of the Mycotoxins

The study of toxinogenesis was made according to the method reported by Khaddor **(Khaddor et al. 2007)**. The chromatography technique of thin layer which was adopted in this study is described by Mills **(Mills et al. 1995)**. The TLC plates used are 60 Kieselguhr F_{254}, mycotoxins standards used by the reference of migration forehead (Rf) were patulin, citrinin, ochratoxin A, penicillic acid and grisefulvin. 10 ml each of ethanol extract and 10 ml of standard solutions (1 mg / ml) were spotted on TLC plates. Elution systems used are: toluene - ethyl acetate - formic acid (5/4/1, v / v / v) and chloroform - acetone - 2-propanol (85/15/20, v / v / v). The plates were examined in daylight and by ultraviolet 365 and 254 nm after spraying the spots by ANIS: 0.5% (v / v) of p-anisaldehyde solution in absolute ethanol – cold acetic acid and concentrated sulfuric acid (17/2/1, v / v / v), 8 min heating to 120 ° C. Some toxins react specifically with ANIS at this temperature, leading to characteristic colors under daylight, namely blue lilac for penicillic acid, yellow for terrestrique acid and gray for aurantiamine.

Results and discussion

Purification and identification of the *Penicillium aurantiogriseum* strain

The purified strain of *P. aurantiogriseum* had the morphological characters summarized in Table2.

Table 2. The morphological characters of the *P. aurantiogriseum*strain

Colony on CYA (25°C)	30-37 mm diam, radially silcate, moderately deep, texture granular ; reverse pale Reverse colour Yellow
mycelium	white
Pigmentation	soluble, brown.
Exudate	pale brown
Smell	heavy
Conidiophores(on MEA)	borne singly or in fascicles, mostly from subsurface hyphae, smooth to finely roughened bearing terminal terverticillate mostly 7-10 µm long
Metulae	spherical, usually 3 – 4 µm long.
Conidia	blue grey conidia on both CYA and MEA

Mycotoxin Production

The chromatographic analysis of the extracts obtained from different culture media of *P. aurantiogriseum* (YES Broth, YES and MEA agar) allowed highlighting three mycotoxins: penicillic acid, terrestric acid and aurantiamin. These mycotoxins have been reported to be produced by this species in previous works **(Colombo et al. 2003; Frisvad and Samson 2004; Khaddor et al. 2007).**

The effect of culture media on the production of mycotoxins

Results of our study showed a great diversity regarding mycotoxin production by *P. aurantiogriseum* in different culture media (Figure 1). Production of penecillic acid was detected in CYA, BM and YES media, while aurantiamin was produced only in MB medium and terrestric acid in YES Broth. Mills et al **(Mills et al. 1995)** have also found penicillic acid and aurantiamin in CYA. However, they have detected terrestric acid in YES agar. This suggests that the culture medium composition has an influence on *Penicillium* toxinogenesis.Some sugars like glucose which are more favorable for the growth of Penicilliums than others (sucrose and fructose) due to their assimilation which is in agreement with other studies that have shown the effect of culture conditions on the excretion of mycotoxins by some strains of *P. Roqueforti* **(Kristian Fog Nielsen 2002; Piva et al. 1976; Ren, Ahearn, and J 1999)**.

The effect of glucose on the production of mycotoxins

We observed a production of aurantiamin in glucose-free BM medium. This production decreases with the increase of glucose concentration in the medium. On the other hand, penicillic acid production increases according to the concentration of glucose in BM medium (Figure 1) which led to conclusion that aurantiamin production is inhibited by glucose. Similarly, many cases of glucose negative effect in the secondary metabolism have been reported **(Gallo and Katz 1972; Hurley and Bialek 1973; Telma Elita Bertolin et al. 2003)**. For example, glucose has proved to negatively affect Ochratoxin A biosynthesis by suppressing the biosynthetic pathway enzymes **(Muhlencoert et al. 2004)**. It has also been reported that glucose inhibits the production of extracellular enzymes and sporulation **(Shafikhani, Nunez, and Leighton 2003)**. Moreover, the addition of glucose suppresses penicillin production in *P. chrysogenum* **(Sanchez et al. 2010).**Some possible explanation is that glucose excess inhibits the synthesis of extracellular enzymes **(Strauch and Hoch 1993a)**, the expression of the Krebs cycle enzymes **(Fouet, et al. 1990)** and the initiating of sporulation **(Takahashi, and MacKenzie, 1981)**. Furthermore, the glucose inhibitory effect in some microorganisms is due to the acidification resulting from to the accumulation of organic acids such as lactic, acetic, citric and pyruvic acids. The fact that penicillic acid production is not affected by glucose concentration increase in the culture medium is may corroborated by the lack of pH influence on the production of this mycotoxin **(Regassa, Novick, and Betley 1992).**

The effect of sucrose on the production of mycotoxins

We observed terrestric acid production in the YES medium. This production increased proportionally to sucrose concentration in the YES Broth, while it does not occur in YES agar. On the other hand, penicillic acid production is inversely proportional to the concentration of sucrose in YES.

Thus, it seems that terrestric acid production is induced by the sucrose. This corresponds with the results of **(Mills et al. 1995)** who reported that terrestric acid is produced in the YES medium in which sucrose is the only carbon source. In contrast, the production of penicillic acid in the YES has decreased. This could be due to the slower catabolism of sucrose used in YES medium and which is due to the absence of the enzyme invertase into strain compared to the catabolism of glucose which is rapidly hydrolyzed in MB medium. However, neither fructose nor a mixture of glucose and fructose reproduces the stimulating effect of sucrose observed in *Fusarium fujikuroi*, suggesting that the induction signal is the sucrose itself, and not any sucrose derivative metabolite **(Jiao, Kawakami, and Nakajima 2008)**. Similarly, it

has been demonstrated that the production of antibiotics and other secondary metabolites are negatively affected when the carbon source used is rapidly assimilated for growth. However, when the carbon source is slowly introduced into the culture, the secondary metabolite is produced efficiently. Although the regulation of carbon source in the biosynthesis of mycotoxins has been recognized since the 1950s, the mechanisms involved are only known in some cases. For example, in the production of actinomycin, the carbon catabolite repression of an enzyme from secondary metabolism is involved. On the other hand, the production of α-lactams by *Cephalosporium acremonium* seems to be affected by some type of inhibition catabolic **(Aharonowitz and Demain 1978).**

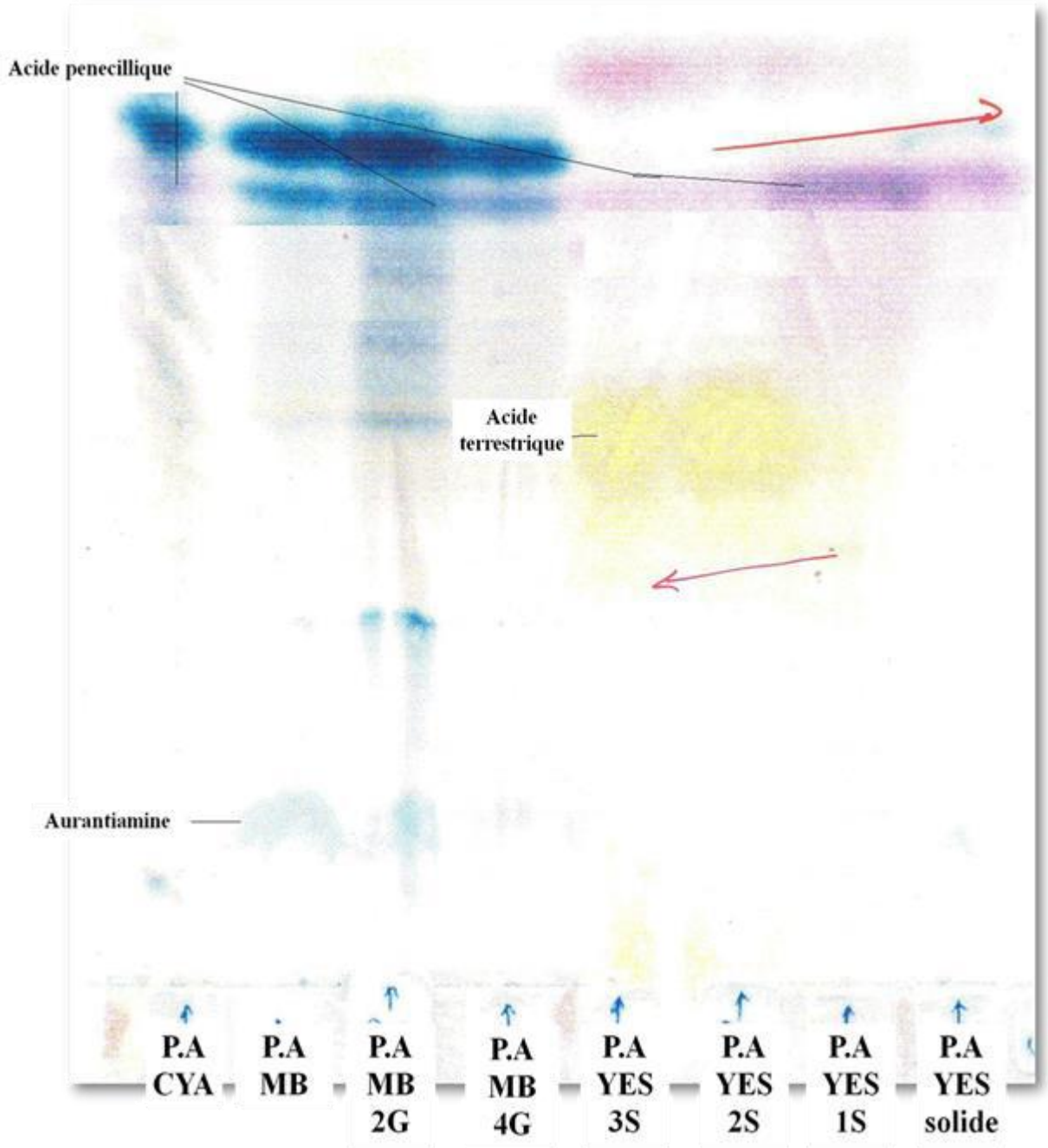

Figure1: mycotoxins on chromatogram *PA* secreted in various culture media and various concentrations of glucose and sucrose (TEF is the migration of solvent)

Conclusions

Several mycotoxins are controlled by carbon sources in culture media. It has been found that the growth of *P. aurantiogriseum* in the medium containing glucose reduced considerably as compared to that observed in the medium containing sucrose **(Oksman et al. 1994).** All this corresponds with our results, where the level of production of metabolites studied in disaccharides was higher than in monosaccharides.

Mycotoxin production by *P. auratiogriseum* is closely dependent on growing conditions. It is difficult to explain metabolically the action of various factors studied.

However, it is interesting to note that factors which could prevent the production of mycotoxins are in the cytoplasm level because all the reactions are reversible. Furthermore, the mycotoxins seem not to be formed if the medium is rich in sugar only, but the production changes with the changes in concentration of the other carbon sources.

Mycotoxin production by *P. aurantiogriseum* also seems to affect the physiological state of its growth and reproduction.

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COMPARATIVE TOXICITY OF GARLIC JUICE AND FENAZAQUIN TO POPULATION OF CITRUS MITES

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Abstract

Several natural products present in plant extracts have been evaluated for their effectiveness as insecticides. The insecticidal properties of Alliaceae have been reported; among these, Garlic and onion, plants with varied biological properties, which contains benefits molecules responsible for pest potential control of alliaceae. The purpose of this study was to compare the effect of chemical and biopesticide on citrus mites, especially *Tetranychus urticae*, *Panonychus citri* and *Eutetranychus orientalis*. Chemical treatment (Fenazaquin) and biopesticide (Garlic juice+Alcohol) applied on this study to control the various stages of mites, have reduced the proliferation of mobile forms and reducing the number of eggs to acceptable levels. The Fenazaquin product is highly toxic against adults and larvae of mites, but with adverse effects on phytoseiid. Garlic juice + alcohol revealed efficiency from 50 to 57,69% against the mobile forms of *T. urticae*, however, it was effective against the motile forms of *P. citri* and *E. orientalis* with an efficiency of 85,71% and 100% respectively, its action has also reduced the number of eggs of *T. urticae* and *E. orientalis* at low levels. Therefore, this biopesticide is technically and economically conceivable as the infestation by mite is low.

Keywords: *Alliaceae, garlic, mites, Fenazaquin, phytoseiid.*

Introduction

Chemical control, sometimes abusive, and considerable advances in agricultural technology to control pests, contributed, among other things, the destruction of useful auxiliary fauna and outbreaks of new pests which phytophagous mites (Nafide M, 2008). The mixed results are likely due to the fact that studies have been conducted on mites at different levels of exposure to insecticides and therefore different levels of resistance (Ako *and al.*, 2006).

The damage caused by these mites are manifested by premature leaf drop sometimes reaching, in heavy infestations, defoliation of the tree, and therefore, impairment of the quality and quantity losses of production (Vacante, 2010). Lower populations in dry areas, can produce the same effect (CABI et OEPP, 1990).

That is why the search for new organic products as an alternative to harmful chemicals on the auxiliary fauna, could remedy the systematic chemical interventions. In addition, the use of different formulations (e.g. azadirachtin) of these biopesticides can induce delayed effects on spider mites (Mansour *and al.*, 1993)

The insecticidal properties of Alliaceae have been reported; among these, Garlic and onion, plants with varied biological properties, which contains benefits molecules responsible for pest potential control of Alliaceae (Arnault and *al.*, 2005).

This study aims to determine and compare the effect of a chemical and a biopesticide on spider mites on *Citrus*, especially *T. urticae*, *E. orientalis* and *P. citri*.

In order to study the possibility of the feasibility for a future substitution of chemicals with biopesticides which are efficient, and accessible to all citrus growers and not harmful to

beneficial fauna, and also authorized by the set of specifications for organic farming. Therefore, the trial was conducted against spider mites in the Field Gazelle-Delassus in the region of Taroudant (Morocco) to assess the effectiveness of products in different stages of these mites.

Material and methods

1. Plot trial

The trial was conducted on June, 2013 in the S1P3 plot, whose characteristics are presented in the following table:

Table 1 : Characteristics of study plot

Plot	**S1P3**
variety	Nour
area (ha)	1,02
Rootstock	Citrange
Planting year	1994
Number of existing trees	656
irrigation type	Drip

The choice of the plot was based on the rate of infestation was more important compared to the other plots.

2. Tested products

The products used are presented in the table below :

Table 2 : Used products

Specialities	**Active ingredient**	**Formulation**	**Doses**	**Action Type**
PRIDE 200 SC	Fenazaquin	SC	40 cc/hl	Contact
Garlic Juice **+ Alcohol	Sulfur	Liquid	625cc of Garlic Juice+16cc of Alcohol	Contact + inhalation

** : 1L of garlic juice is obtained after maceration of 500 g of garlic in a liter of water.

3. Experimental plan

To perform this test, authors opted for an experimental randomized complete block, consisting of 3 blocks (3 repetitions). Each block is divided into three basic plots. The treatment assignment was made randomly. To avoid the effect of the treatments, the blocks are separated by a passage 4 m, while for basic plots, we opted for a line of trees separating two elementary plots (Figure 1).

Each elementary plot consists of 18 trees, so we treated 72 trees.

The treatments were conducted on June 6 at 7:30 am (this is the week that follows the maximum infestation by mites). In fact, we started with the control treatments (water), then the biopesticide treatment: Garlic Juice**+Alcohol, and chemical treatment: Fenazaquin.

The experimental plan is performed as follows :

: **Trees treated with chemical (Fenazaquin)**

: Trees treated with biopesticide (Garlic juice+Alcohol)

: Control trees (water)

Figure 1 : Experimental plan of test

4. Treatment equipment

Applications made on citrus were applied with a sprayer towed with projected jets, with a 2000L cistern and two spears. Spraying was done with a pressure of 30 bar. The porridge used is 12 liters per tree, allowing the wetting of all the parts of the tree.

5. Sampling technique and notations

Sampling consist to assess the number of living mites on 30 leaves for each treatment. In each elementary plot, a single tree and a number of leaves have were the object of our sampling. The sampling method in each elementary plot is as follows: 6 leaves per tree, 2 within the canopy on twig and 4 to the different cardinal points of the tree.

The evaluation is to determine the level of infestation of each elementary plot, to count individuals per species per stage and determine the effects on acariphage fauna. The Calculating of the effectiveness of tested products, in the various control dates was performed using the ABBOT's method :

$$\text{Effectiveness\_ABBOT} = 100 \times (T_0 - T_t)/T_0$$

T_t= Number of mites in the treated plot

T_0= Number of mites in the control plot

We can classify the products from the values of the effectiveness of each product as follows :

Table 3 : Effectiveness class

% EFFECTIVENESS	CLASS
95 à 100%	very good efficacy
80 à 95%	good efficacy
60 à 80%	average efficacy
40 à 60%	low efficacy
40%	Efficacy with no practical interest

6. Frequency

Samples taken before and after treatment are seven, spread over time, as shown in the following table :

Table 4 : Frequency of samples collection

Counting	Frequency
1	just before the treatment
2	3 days after treatment
3	7 days after treatment
4	10 days after treatment
5	13 days after treatment
6	16 days after treatment
7	19 days after treatment

Results and discussion

1. Infestation rate and demographic structure of the mites population

T. urticae

Situation before treatment

Just before the treatment, the infestation rate of leaves was about 12%, the statistical analysis of the data after the angular transformation, showed no significant effect between the elementary plots receiving the various treatments applied in terms of infestation rates. Analysis of the variance of the density of mobile forms and Eggs has also showed no significant effect at the 5% level between the different treatments. Furthermore, statistical analysis of the density of larvae and eggs of *T. urticae* revealed no significant effect at the 5% level between the different treatments.

3 days after treatment

Statistical analysis of the data collected revealed a significant effect of treatment applied ($p = 0.001 < 0.05$).

The statistical processing of the variable number of mites per leaf, did not record a significant treatment effect ($p = 0.419 > 0.05$). Garlic has registered the lowest rate of infestation with 0%. The fenazaquin recorded 3.33% as infestation rate. For control treatment, the rate infestation was 6.67%. Analysis of variance of the variable number of *T. urticae* larvae showed no significant effect of treatment at 5% level ($p = 0.328 > 0.05$). The analysis of variance of the variable number of eggs of *T. urticae* reported no significant effect of treatment ($p = 0.256 > 0.05$).

7 days after treatment

The statistical data processing of the variable the infestation rate of leaves showed a highly significant effect of treatment at 5% level ($p = 0.000 < 0.05$).

The comparison of averages with the SNK test identified two homogeneous groups. Indeed, the first group containing the fenazaquin with law infestation rate of 0% and the second group containing the control treatment and garlic with an infestation rate of 3.33% and 6.67% respectively.

Nevertheless, the comparison of averages by SNK test at 5% of the number of mobile forms (adults and larvae) and eggs of *T. urticae* revealed a single homogenous group.

10 days after treatment

The statistical data processing of this sampling, reported a highly significant effect of treatment ($p = 0.000 < 0.05$).

Garlic marked the lowest rate of infestation with 0%, while fenazaquin recorded an infestation rate of 3.33%, and finally the control treatment whose infestation rate was 6.67%.

13 days after treatment

During the sampling, analysis of the data collected revealed a significant effect of treatment at 5% level ($p = 0.000 < 0.05$). The infestation rate of fenazaquin and garlic was 0%, and 6.67% for control treatment.

16 days after treatment

During this sampling, statistical analysis of the infestation rate variable showed a significant effect of the treatment at 5% level ($p=0.000<0.05$). The infestation rate of fenazaquin, garlic and control treatment was respectively 0%, 3.00% and 10%.

Statistical analyzes of the variables number of adults, number of larvae and number of eggs/30 leaves reported a significant effect of treatment for only the variable number of adults/30 leaves ($p=0.041<0.05$).

P. citri

Situation before treatment

Statistical analysis of the infestation rate and density of mobile forms of *P. citri* showed no significant effect at the 5% level between the 4 treatments. The infestation rate was 5%.

3 days after treatment

The analysis of variance showed that there was a highly significant effect of treatment ($p = 0.000 < 0.05$). During the sampling, 0% was noted for garlic juice and fenazaquin as infestation rate, while 10% was recorded for the control treatment.

However, the data processing of the number of mobile forms on infested leaves showed that there was no significant effect of treatment ($p = 0.186 > 0.05$).

7 days after treatment

During this control, the infestation rate was 0% for each treatments applied.

The comparison of averages by SNK test at 5% of number of mobile forms of *P. citri* has unveiled a single homogeneous group.

10 days after treatment

The analysis of statistical data by SNK test of the variable infestation rate of *P. citri* was almost similar to that of *T. urticae*.

Garlic recorded 0% as infestation rate, while the fenazaquin and the control treatment noted an infestation rate of 3.33%.

13 days after treatment

The analysis of variance of the variable rate of infection for the species of *P. citri* showed a highly significant effect of treatment at 5% level ($p = 0.000 < 0.05$).

On the other hand, the analysis of variance of the variable number of mobile forms/30 leaves demonstrated that the treatment has no significant effect at 5% on the number of mobile forms of *P. citri* ($p = 0.053 > 0.05$). In addition, the comparison of averages by the test revealed a significant difference between the fenazaquin and the control treatment ($p = 0.031 < 0.05$).

16 days after treatment

The analysis of variance of the variable infestation rate of *P. citri* noted a significant effect of treatment ($p=0.000<0.05$). Indeed, the rate of infestation in the control treatment was 6.67%, but, other treatments reported 0% as infestation rate.

The table 5 shows the average densities of mobile forms for different treatments, and therefore, we find that Garlic recorded the lowest average density, followed by fenazaquin, and the control treatment which the average density is higher compared to other treatments. That reveals the toxic effect of garlic on mobile forms, those results are concorded with those obtained by El Hardouni (2006).

E. orientalis

Situation before treatment

Before applying treatment, statistical analysis showed no significant effect at 5% for mobile forms and eggs, the infestation rate was 2%. Therefore, the number of *E. orientalis* appears too low compared to other species.

3 days after treatment

During this control, the analysis of variance of the variable infestation rate of leaves showed a highly significant effect of treatment ($p = 0.001 < 0.05$). Therefore, the comparison of averages by SNK test revealed two homogeneous groups. The first group containing the fenazaquin with an infestation rate of 0% and the second group with Garlic and control treatment with an infestation rate of 3.33% and 6.67% respectively.

The analysis of variance of the variable number of mobile forms and eggs showed no significant effect of treatment at 5% level ($p = 0.431 > 0.05$, $p = 0.108 > 0.05$; $p = 0.691 > 0.05$).

7 days after treatment

The analysis of variance of the variable rate of infestation noted a highly significant effect of treatment ($p = 0.000 < 0.05$).

Nevertheless, by the analysis of variance densities adults, larvae and eggs showed no significant effect of treatment at 5%. For biopesticide, the rate of infection was 3.33%. For fenazaquin and the control treatment, 0% is recorded as infestation rate.

10 days after treatment

The infestation rate of *E. orientalis* was 0% for all treatments, the plots treated with the fenazaquin, no activity of mobile forms or eggs have been reported on samples.

13 days after treatment

The infestation rate of *E. orientalis* was the lowest compared to other species, with an average of 0% for all treatments. Furthermore, analysis of variance of the variable number of mobile forms (adults and larvae) per 30 leaves reported no significant effect of treatment [(p (adults) $=0.534>0.05$) ; (p (larvae) $=0.574>0.05$)]. However, analysis of variance of the variable number of eggs per 30 leaves noted a significant effect of treatment ($p=0.029<0.05$).

The samples examined from the plots treated by fenazaquin, revealed no presence of *E. orientalis*.

16 days after treatment

The samples including the control treatment didn't detected presence of mobile forms of *E. orientalis*, it may be due to the high temperature caused mortality of mobile forms during this sampling day (36 ° C).

19 days after treatment

During the last sampling, the infestation rate of species *T. urticae*, *P. citri* and *E. orientalis* was 0% for all treatments including the control treatment because of the high temperature (38 ° C) whose thermal effect was unfavourable for the evolution of the population of mites.

Spevies	Stages	Treatment	Before treatment (J)	J+3	J+7	J+10	J+13	J+16	J+19
T. urticae	**Adults**	**Fenazaquin**	1.20[a]	0.20 ± 0.17[a]	0.033 ± 0.03[a]	0.30 ± 0.21[a]	0.033 ± 0.03[a]	0.033 ± 0.03[a]	0.00 ± 0.00[a]
		Garlic	1.00[a]	0.17 ± 0.07[a]	0.57 ± 0.36[a]	0.10 ± 0.07[a]	0.13 ± 0.06[a]	0.37 ± 0.24[ab]	0.133 ± 0.13[a]
		Control	1.10[a]	0.73 ± 0.42[a]	0.43 ± 0.27[a]	0.53 ± 0.27[a]	0.43 ± 0.28[a]	0.87 ± 0.36[b]	0.00 ± 0.00[a]
	Larvae	**Fenazaquin**	0.22[a]	0.00 ± 0.00[a]	0.033 ± 0.03[a]	0.033 ± 0.03[a]	0.033 ± 0.03[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		Garlic	0.20[a]	0.00 ± 0.00[a]	0.10 ± 0.07[a]	0.00 ± 0.00[a]	0.20 ± 0.11[a]	0.033 ± 0.03[a]	0.00 ± 0.00[a]
		Control	0.24[a]	0.20 ± 0.17[a]	0.033 ± 0.03[a]	0.033 ± 0.03[a]	0.13 ± 0.08[a]	0.07 ± 0.05[a]	0.00 ± 0.00[a]
	Eggs	**Fenazaquin**	0.93[a]	0.20 ± 0.20[a]	0.83 ± 0.71[a]	1.23 ± 0.75[a]	0.17 ± 0.14[a]	0.07 ± 0.05[a]	0.00 ± 0.00[a]
		Garlic	0.91[a]	0.10 ± 0.07[a]	0.80 ± 0.65[a]	0.27 ± 0.15[a]	0.47 ± 0.35[a]	0.30 ± 0.21[a]	0.00 ± 0.00[a]
		Control	0.95[a]	0.57 ± 0.29[a]	0.30 ± 0.13[a]	1.07 ± 0.64[a]	0.83 ± 0.36[a]	0.37 ± 0.21[a]	0.00 ± 0.00[a]
P. citri	**Mobile forms**	**Fenazaquin**	0.40[a]	0.07 ± 0.05[a]	0.033 ± 0.03[a]	0.30 ± 0.21[a]	0.033 ± 0.03[a]	0.07 ± 0.05[a]	0.00 ± 0.00[a]
		Garlic	0.36[a]	0.07 ± 0.05[a]	0.10 ± 0.06[a]	0.20 ± 0.07[a]	0.10 ± 0.07[a]	0.033 ± 0.03[a]	0.033 ± 0.03[a]
		Control	0,38[a]	0.33 ± 0.17[a]	0.10 ± 0.06[a]	0.33 ± 0.16[a]	0.40 ± 0.18[a]	0.23 ± 0.11[a]	0.00 ± 0.00[a]
E. orientalis	**Adults**	**Fenazaquin**	0.18[a]	0.033 ± 0.03[a]	0.033 ± 0.03[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		Garlic	0.20[a]	0.17 ± 0.11[a]	0.40 ± 0.33[a]	0.10 ± 0.06[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		Control	0.16[a]	0.20 ± 0.14[a]	0.033 ± 0.03[a]	0.10 ± 0.07[a]	0.033 ± 0.03[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	Larvae	**Fenazaquin**	0.07[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		Garlic	0.05[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.033 ± 0.03[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		Control	0.03[a]	0.13 ± 0.09[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	Eggs	**Fenazaquin**	1.26[a]	0.20 ± 0.12[a]	0.40 ± 0.23[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		Garlic	1.22[a]	0.57 ± 0.23[a]	0.133 ± 0.13[a]	0.67 ± 0.28[a]	0.57 ± 0.29[b]	0.033 ± 0.03[a]	0.10 ± 0.10[a]
		Control	1.24[a]	0.83 ± 0.61[a]	0.033 ± 0.03[a]	0.70 ± 0.70[a]	0.07 ± 0.05[a]	0.53 ± 0.33[a]	0.00 ± 0.00[a]

Table 5 : Average densities of each stage of mite species collected during the test period

2. Comparison of the products tested efficacy

Te examination of Figure 2 allows that the evolution of the effectiveness of the tow products is increasing from the application of the treatment. Indeed, the effect of the products evolves gradually from 77.27% and 72.72%, recorded on J + 3, respectively for Garlic and fenazaquin to 57.69% and 96.15% considered on J + 16. This biopesticide was tested and reduced the average level of the population of *T. urticae* in peach orchard (Sagouti, 2007). As for *T. urticae* larvae (Figure 3), the efficacy of product follows a fluctuating evolution, However, the effect increased from 100% to 100% on J + 3 for Garlic and fenazaquin until 50% and 100% on J + 16. In addition, garlic has shown an average efficacy against adult and very effective against the larvae, while fenazaquin was very effective against mobile forms almost along the trial period.

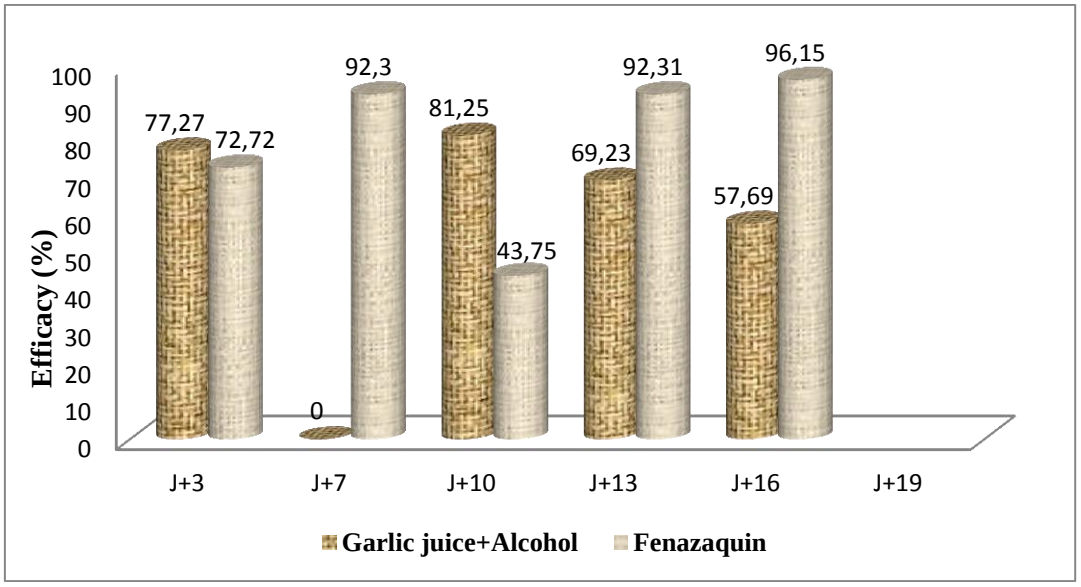

Figure 2 : Evolution the effectiveness of the products tested on adults of *T. urticae*

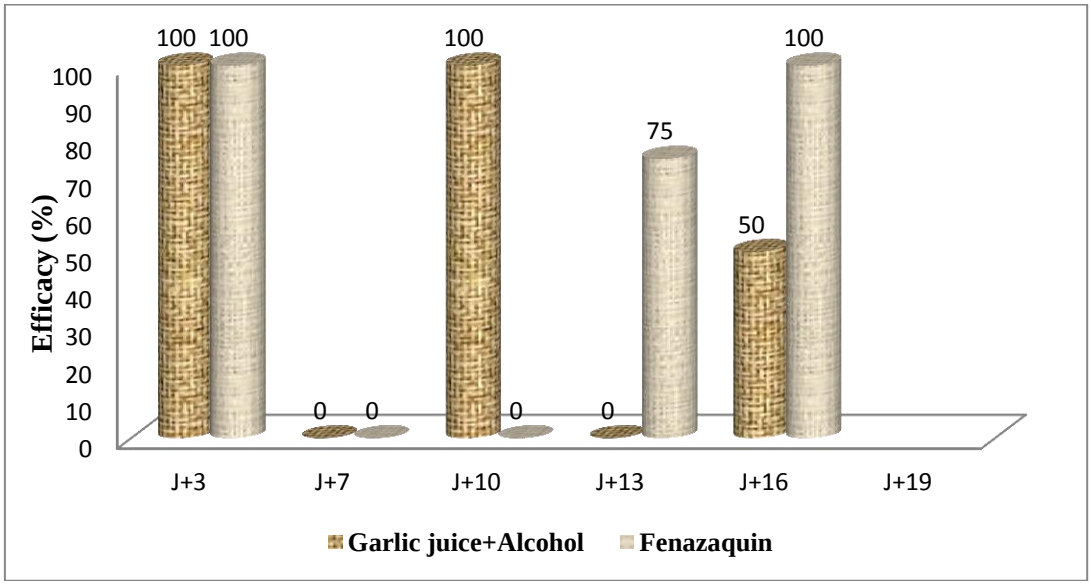

Figure 3 : Evolution the effectiveness of the products tested on larvae of *T. urticae*

Regarding the species *P. citri* (Figure 4), Garlic unveiled an efficacy of 85.71% in the 1st day of sampling after treatment (J + 3) and J + 16, and therefore, it is good efficacy against the

mobile forms of *P. citri*. The fenazaquin has good efficacy, since it reached 100% in the last day of sampling (J + 19). Studies in the Gharb region (Morocco), have indicated that the use of garlic juice and alcohol can replace the chemicals to treat against spider mites (*T. urticae* and *P. citri*) on citrus, knowing that biopesticide is conceivable in technical and economic perspective, authorized by the specifications of organic farming (El Hardouni, 2006; Boutaleb, 2011).

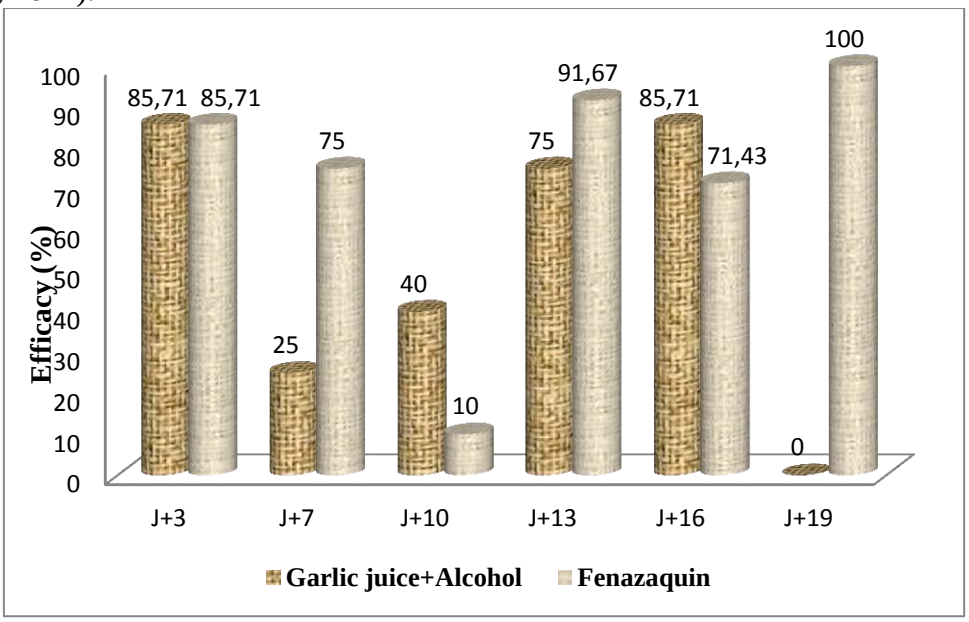

Figure 4 : Evolution the effectiveness of the products tested on *P. citri*

The figure below illustrates the effectiveness of both products against *E. orientalis*, the fenazaquin has noted very good efficacy, which is increased from 83.33% (J + 3) to 100% (J + 19). These results are in agreement with those found by Abbasi (2011), he noted that fenazaquin has an average or very good efficacy, and suggested washing the trees with water + potassium soap (repeat according to the infestation level).

As for garlic, the effectiveness of this biopesticide was very low on J + 3 (16.67%), but it revealed a very good effect on the last sample (100%).

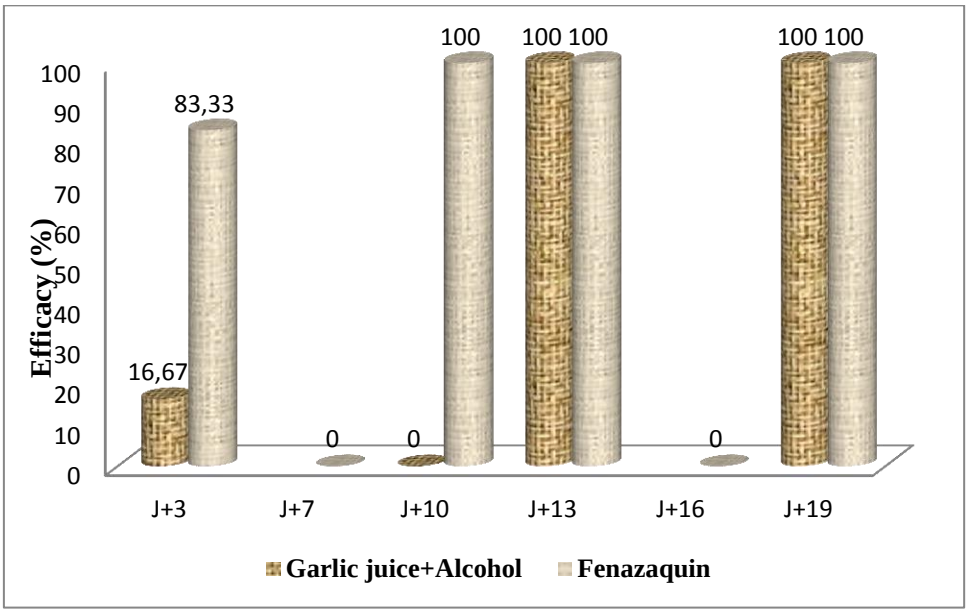

Figure 5 : Evolution the effectiveness of the products tested on *E. orientalis*

Conclusion

Chemical treatment (fenazaquin) and biopesticide (Garlic + alcohol) applied in the test, against the different stages of mites namely *T. urticae*, *P. citri* and *E. orientalis*, have reduced the proliferation of mobile forms and reducing the number of eggs at low levels compared with the control treatment. The fenazaquin assured very good control (100%) of adults and larvae of mites, with adverse effects on auxiliary fauna. The garlic juice showed a weak effect against the mobile forms of *T. urticae*, but, it appears effective against mobile forms of *P. citri* and *E. orientalis*. In the other hand, it's action reduced also the number of eggs of *T. urticae* and *E. orientalis* at low levels. Garlic juice + alcohol provides an average efficiency against mobile forms of *E. orientalis*, but with weak effect on the mite eggs in heavy infestations.

The application of biopesticides treatment is recommended for low infestations of mites for better control, and therefore, they can be used as an alternative means of control to chemicals, knowing that biopesticides are biodegradable in nature, and also technically and economically conceivable. In addition, other biological controls are possible and may be associated with the biopesticides such releases of predators.

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MICROBIOLOGICAL DRESSING OF PEA SEEDS AS A FORM OF INCREASE RESISTANCE AND PLANT DEVELOPMENT

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Abstract

The duty of the integrated protection of plants was introduced in Poland in 2014 and it is related with the application of biological and other non chemical methods of plant protection. Health and quality of seed is extremely important, especially in organic farming. Seeds treatments before sowing e.g. dressing with the microorganisms can stimulate their germination, the health of the seedlings and to improve the development of young plants. The search for alternative means and methods to seeds dressing was the purpose of this research. A seed dressing in dry and wet way was also evaluated for finding the most effective one. The experiments were conducted in greenhouse and field conditions with pea (*Pisum sativum* L.) seeds. Commercial products based on *Pythium oligandrum* Drechsler, *Trichoderma asperellum* Samuels, Liechfeldt et Nirenberg and *Saccharomyces cerevisiae* Meyen ex E.C. Hansen in doses of 10 g/1 kg of seeds were used. Also the product containing a complex of microorganisms (EM Farm) in dose 1 ml/100 ml of water was included. The number of seedlings, health and development of plants according to microbial dressing was evaluated. In glasshouses conditions it was found that *T. asperellum* and *P. oligandrum* the most improved the number of plants emergence, healthiness and development of pea seedlings. Dry seed treatment was more effective compared to wet dressing way. In the field conditions *T. asperellum* was the most effective. *P. oligandrum* had slightly positive effect only on number of leaves and development of the root. *S. cerevisiae* was the least effective in the promotion of development of the seedlings.

Keywords: *organic farming, Pythium oligandrum, seed dressing, Trichoderma asperellum, yeast*

Introduction

The soil environment is the habitat of a lot of microorganisms. The research on the application of antagonistic microorganisms and organic compounds in controlling plant diseases was developed worldwide (Martin and Hancock 1987, Orlikowski and Skrzypczak 2003, Pięta et al. 1998). Introducing biopreparations in plant protection can contribute to reduced use of fungicides, and – as a result – less contamination of the natural environment (Pięta et al., 2005). Plant biostimulants include diverse substances and microorganisms that can enhance plant growth. The global market for biostimulants has been projected to reach $2.241million by 2018 and to have a compound annual growth rate of 12.5 % from 2013 to 2018 (Anonymous, 2013). According to the same study, the largest market for biostimulants in 2012 was Europe. The European biostimulants industry council (EBIC) reported that in 2012 over 6.2 million hectares were treated with biostimulants (European Biostimulants Industry Council 2013). (Calvo et al., 2014).

Biological control of plant diseases is the utilization of one or more living organisms which have antagonistic effect against various plant pathogens to achieve the suppression of plant diseases. Health and quality of seed is extremely important. Seed treatment can stimulate their germination, emergence and improve the health parameters of development of young plants. It is a necessary condition for obtaining high plant productivity, and thus one of the cheapest means of determining the good performance of crops. The most important enriching

treatments should be dressing (Orzeszko-Rywka et al. 2011). In organic farming chemical seed treatments that are very effective in the protection of seeds and seedlings are not permitted. Sowing seeds without dressing is quite risky, may lead to a mass loss of plants in the early stages of development and consequently, to lower yield and deterioration of its quality. Therefore, in many research centers are developed methods of alternative and acceptable in the organic farming ways of dressing seeds (Orzeszko-Rywka, 2011). The aim of the study was to choose methods of microbiological pea seed treatment to accelerate the emergence and improve the development seedlings and young plants. The glasshouse trials were performed in 2014, the field experiments were conducted in 2015.

Materials and methods

The glasshouse is located near institute in Poznan, the Field Experimental Station of IPP-NRI is located in the Western part of Poland. Commercial products based on *Pythium oligandrum* (Polyversum), *Trichoderma asperellum* (Trifender), *Saccharomyces cerevisiae* (baker's yeast) in doses of 10 g/1 kg of seeds were used. Each experimental combination included 4 repetitions (big plots). Product Polyversum is registered in Poland as biological plant product and one gram of it consists $1x10^6$ of zoospores *P. oligandrum*. Trifender WP (product of Biovéd, Hungary) is used in Poland as microbial plant growth promoter. One gram of the product contained $5x10^8$ of conidium isolate T1. As source of *S. cerevisiae* trade bakery product with lyophilized fungus in concentrate $11x10^6$ of *S. cerevisae*in in one gram was used.

Glasshouse conditions

Combinations with each microorganism were made with 100 seeds (5 seeds in 20 pots), which were sown in the sterile perlit or soil. Pots sowed seeds were kept in a greenhouse at 20 ° C giving optimal growth of seedlings. For dry dressing seeds were placed in a Petri dish and mixed with microbial product in accurate dose in relation to weight of seeds. Petri dishes were shaken by 10 s and after next 5 minutes seeds were sown. In way of wet dressing the fixed dose of Polyversum was mixed with warm water (100 ml). Seeds were put into solution for 0.5 h. EM Farm in dose 1 ml/100 ml water was also used. Dressed seeds were out and slightly drained on paper towel by 24 hrs. Two varieties of pea were used, “Sześciotygodniowy” and “Cud Kelvedonu”. In glasshouse conditions the number of health and dead seedlings (infected by *Fusarium* spp., *Rhizoctonia solani*) during 2 weeks after sowing was evaluated. After 4 weeks, the seedlings were measured (fresh weight of the total plant, weight of the roots and number of leaves).

Field conditions

The experiment was established using the random block design on plots of 400 m^2 area where pea cv. Milwa was sown. The field experiments were performed in 2015. Date of sowing was 7 April, number of plant emergence was calculated in 16 April, on 1 m in four locations in each combination. Seeds were treated with powder of each used microbial product, only EM was used as water treatment. These same doses as in glasshouse were used.

Statistical methods

Differences between treated and untreated were determined using Tukey’s multiple range test at $P \leq 0.05$ for selected combination. The data are presented as untransformed means.

Results and discussion

Trichoderma isolates are known for their ability to control plant pathogens and have been used in different fields of production and protection in agriculture (Howell, 2003; Kowalska, 2010). *Trichoderma* is able not only to produce toxic compounds with a direct antimicrobial activity against pathogens, but also generates fungal substances which are able to stimulate

plant to produce its own defense metabolites. An additional category of inoculants is the product referred as EM “effective microorganisms.” EM consist of around 70 species of microorganisms belonging to five groups, namely lactic acid bacteria, photosynthetic bacteria, actinomycetes, yeast fungi and filamentous fungi (Valarini et al. 2003). The application of EM has a beneficial effect on soil texture and quality (Khaliq et al. 2006). In Poland the biopreparation EM is registered as a soil enhancer recommended for use in organic farming (Okorski et al. 2008). As described by Hu and Qi (2013), the inoculant called EM was first described in non-refereed presentations in the early 1900s. The contents of EM were subsequently summarized (Hu and Qi 2013) The microbes are fermented together in the presence of organic wastes and molasses (Khaliq et al. 2006). Field experiments with cotton showed that EM increased the efficiency of mineral and organic fertilizers, and the combination of EM with organic matter increased yields 23 % compared to treatment with organic matter alone (Khaliq et al. 2006). In contrast, (Mayer et al. 2010) found that EM did not increase yield or soil quality in trials conducted over 4 years in Central Europe. On the basis of data included in table 1 can be stated that that positive effect on number of emergence was confirmed in EM combination inof conducted trials in the glasshouse.

Table 1. Pea seed wet dressing with EM – impact on plants emergence, cv. „Sześciotygodniowy” in glasshouses conditions

Sowing into pots with soil	Treated with EM Number of seedlings	Untreated Number of seedlings
5th day after	29	19
6th day after	39	27
7th day after	54	38
8th day after	61	45
9th day after	62	47

Comparing two methods of dressing can be decided that dry seed treatments were more effective than wet dressing, especially in influence on increase number of seedlings. Our results are presented in Table 2.

Table 2. Pea seed dressing with fungus P. *oligandrum* in different ways, cv. “Cud Kelvedonu” in glasshouses conditions

Sowing into pots with soil	Product Polyversum (10 g/1 kg of seeds)	
	Dry dressing	Wet dressing
number of plants emergence	82 pcs	69 pcs
number of infected plants	5 pcs	5 pcs
mean mass of plant	4.7 g	3.5 g
mean mass of roots	0.6 g	0.4 g

Impact of dry dressing with different microorganisms on cv. “Cud Kelvedonu” in glasshouses conditions was evaluated. Obtained results in this experiment are included in the Table 3.

Table 3. Pea seed dry dressing– impact on plants development, cv. „ Cud Kelvedonu ” in glasshouses conditions

	Perlit				**Soil**			
Microbial agent	number of plants emergence (pcs)	number of infected plants (pcs)	mean mass of whole plant (g)	mean mass of roots (g)	number of plants emergence (pcs)	number of infected plants (pcs)	mean mass of whole plant (g)	mean mass of roots (g)
S. cerevisae	82	4	1.5	0.3	**82**	1	1.3	0.2
Untreated	92	2	1.5	0.3	63	1	1.7	0.4
P. oligandrum	80	2	1.4	0.2	**82**	5	**4.7**	**0.6**
Untreated	92	4	1.2	0.2	73	5	1.0	0.2
T. asperellum	**90**	1	**1.7**	0.4	**80**	1	**1.3**	0.1
Untreated	68	4	1.3	0.4	63	3	1.0	0.1

Fungi of the genus *Trichoderma* are often used as seed treatments influencing favorably the characteristics of seedlings (Sadowski et al., 2005; Mastouri et al., 2010). It is believed that such treatments are favorably only short time and few further reports of Trichoderma impact on plants during their development are available. Our results presented in table 3 clearly points out that effects of seed treatments are more visible in the experiments with soil than in the perlit. The best effect for development of young plants was observed for *T. asperellum* and *P. oligandrum*.
Seed microbial dressing was factor which determined the number of plant emergence in the field conditions. The highest number was noted after treatments with *T. asperellum* and *S. cerevisiae*, (av. 16 pcs/1m). After EM and in plot with untreated seeds were noted 15 pcs/ m, respectively. The lowest number was observed in case of *P. oligandrum* (13 pcs/ m). Effect of dressing seeds compared to untreated seeds is not clear visible and significant.

In field conditions was evaluated the influence of selected microbial products on the development of seedlings. Statistically a confirmation of the effectiveness of certain products was done. Data are presented in the Table 4.

Table 4. Mean values of development parameters of plants collected from different combination in the field conditions

Microbial dressing	weight of whole plant [g]	weight of roots [g]	number of leaves [pcs]
Trichoderma asperellum	**1.28 a**	**0.30 a**	**5.25 a**
Pythium oligandrum	1.05 b	0.10 b	**5.30 a**
Effective microorganisms (EM)	1.20 a	0.25 a	**5.45 a**
S. cerevisae	0.66 c	0.11 b	**4.50 b**
untreated	1.07 b	0.22 a	4.25 b

Values with letters are statistical different within column

In the field conditions seed treatments with *T. asperellum* had the most positive effect on development of seedlings and young plants for all evaluated parameters. EM increased the development of the root part. All microbial except yeast treatments were influencing on increase of number of leaves. In combination with *S. cerevisae* some parameters of

development were worst than in untreated plants. The studies conducted by Pięta et al. (2005) in a growth chamber showed the protective effect of Polyversum used as seed dressing for common bean and pea. Eight fungi species were considered for pea. The seeds were dressed with Polyversum in the dose of 1 g/100 seeds. Five weeks after the experiment was established the number of plants and their healthiness. Fact was confirmed that antagonistic microorganism (*P.oligandrum*) has protective effect. Sobolewski et al. (2013) showed that the carrot seed dressing with selected isolates of the fungus *Trichoderma* spp. against damping-off seedlings significantly reduced the infection of germinating seeds and carrots seedlings by the pathogenic fungus species *Pythium* spp. The use of *Trichoderma* spp. was more efficiently affected the height and weight of the tested carrot seedlings as compared to untreated seed. No effect of phytotoxicity of *Trichoderma* spp. isolates was observed (Sobolewski et al., 2013).

In other tests symptoms of downy mildew (*Peronospora viciae*) on pea leaf, Ascochyta pod spot and Fusarium root significantly was affected by dressing of seeds with EM in 0.2% concentration of solution. (Okorski et al. 2008). EM reduced the incidence of pea diseases. Foliar application of EM significantly increased the rate of photosynthesis in pea. Soil application of EM, seed dressing and chemical control decreased the rate of photosynthesis in pea. Seed dressing with EM and chemical products caused a significant decrease in molar transpiration values in pea. The occurrence of downy mildew of peas significantly reduced the rate of photosynthesis. In studies of Zbroszczyk and Kordas (2012) was the assessment of a potential effect of tillage systems and EM treatments (seed dressing and soil application) on reduction of plant diseases infection. The three-year studies did not show any explicit positive effects of EM on health status of spring wheat grown in a short-term monoculture. Dłużniewska (2006) was conducted the study on the effect of fungicides in concentrations 10 ppm and *T. harzianum, T. pseudokoningii* and *T. viride* usability for biological pea seed treatment against diseases. It was found that seed dressing with *Trichoderma* fungi improved healthiness of pea seedlings threated with diseases. Fungicides have a negative effect on the protective activity of the studied antagonistic fungi (Mastouri et al.,2010). The efficacy of application of Trichoderma was evaluated against wet root rot of chickpea. Seed treatment formulations of Trichoderma species was more effective than soil application for all the parameters. Both soil application and seed treatment formulations enhanced the growth of the plants indicating growth promoting ability of the isolates used for development of the formulations. (Dubey et al., 2012). Polyversum was used among others products in the tests conducted by Horoszkiewicz et al. (2012) to dressing seeds of different varieties of blue lupine, yellow lupine, and pea. Grapefruit extract, garlic pulp and a synthetic seed dressing Vitavax 200 FS were used. No significant differences in the number of infected plants of lupine and the level of infestation between tested biopreparations and control were found. Antagonistic fungi such as *Trichoderma harzianum*, *T.* v*iride* and *T. hamatum, Bacillus subtilis, Pseudomonas fluorescens* and *Saccharomyces cerevisiae* were tested in dose $2x10^4$cfu/mL by El-Mougy et al. (2012). All used seed dressing treatments reduced significantly root rot incidence at both pre-, and post-emergence growth stages of cucumber, cantaloupe, tomato and pepper plants comparing with untreated check control.

On basis gained results we can conclude that in the glasshouses conditions *Trichoderma asperellum* and *Pythium oligandrum* the most improved the number of plants emergence, healthiness and development of pea seedlings, especially in the soil compared to the perlit. Dry seed treatment was more effective compared to wet dressing way. In the field conditions *T. asperellum* was the most effective. *P. oligandrum* had slightly positive effect only on number of leaves and development of the root. *S. cerevisiae* was the less effective in the promotion of development of the seedlings.

Acknowledgement

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Original scientific paper
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EFFICIENCY OF REGULATORS OF GROWTH AND MICROFERTILIZERS ON THE LETTUCE A GRADE GEYSER CULTIVATED BY METHOD OF A FLOWING HYDROPONICS

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Abstract

In the protected ground use of regulators of growth and microfertilizers is mandatory agronomic reception at cultivation of vegetable cultures. In recent years, the market of agrochemical production is filled up annually by new natural and synthetic agrochemical preparations: regulators of growth and microfertilizer, the direction of action and which efficiency aren't always justified in production. Purpose of researches: to study efficiency of regulators of growth (Narcissus, Zircon) and microfertilizers (Siliplant and Rexolin of APN) on productivity, removal of elements and some indicators of quality of on the lettuce a grade Geyser cultivated by method of a flowing hydroponics. Researches on use of regulators of growth and microfertilizers on plants of salad were carried out under production conditions by LLC "Tchaikovsky greenhouses" of Tchaikovsky of the region of Perm Krai in 2010. Lettuce cultivated by method of a flowing hydroponics. It was established that the strait of substrate solution of the drug Narcissus, Zircon, Rexolin APN increases of the salad productivity on 5.20, 10.8 and 3.60 g with five plants, respectively. Effective action from use of microfertilizer of Siliplant on salad it isn't revealed, on the contrary established that the strait of substrate solution led to essential decrease in productivity. A regulator of growth and microfertilizer has no essential impact on the content of nitrogen, phosphorus, calcium, magnesium, ashes (Ffact. < Ftheor.) and on carrying out of removal of elements salad. When using regulators of growth is noted the tendency to decrease in nitrates in sheet lettuce a grade Geyser.

Keywords: *Growth stimulants, micronutrient fertilizers, lettuce a grade Geyser, yield, elemental composition*

Introduction

Protected ground is currently developing as a dynamic and competitive agriculture industry capable of year-round supplying the population with fresh vitamin-rich vegetables. Growing of vegetables by flowing hydroponics method using a complex of chemistry, biology and electronics is a cutting edge direction in a glasshouse industry (Antipova, 2003, 2010).

It is well known, that certain chemical substances used in low concentrations show the positive effect on plant resistance to diseases and stress situations, the seed germination and plant establishment, the stimulation of growth and hasten ripening, the yield and quality of cultivated plants. In protected ground, the use of growth regulators and microfertilizers is mandatory (Antipova, 2003, 2010). In recent years, the market for agrochemical products is annually replenished with new natural and synthetic agrochemical formulations such as growth regulators and microfertilizers, the direction of action and the effectiveness of which are not always justified for production.

The purpose of the research is to study effectiveness of action of growth regulators Narcissus and Zircon and microfertilizers Siliplant and Rexolin APN on yield, removal of the fertilizer elements, and some indicators of the quality of Geyser lettuce grown by method of flowing hydroponics.

Materials and methods

The method of the research: Studies on the use of growth regulators and microfertilizers in plants of lettuce were conducted under production conditions of "Tchaikovsky greenhouse" LLC in Tchaikovsky district of the Perm Krai (Fig. 1)

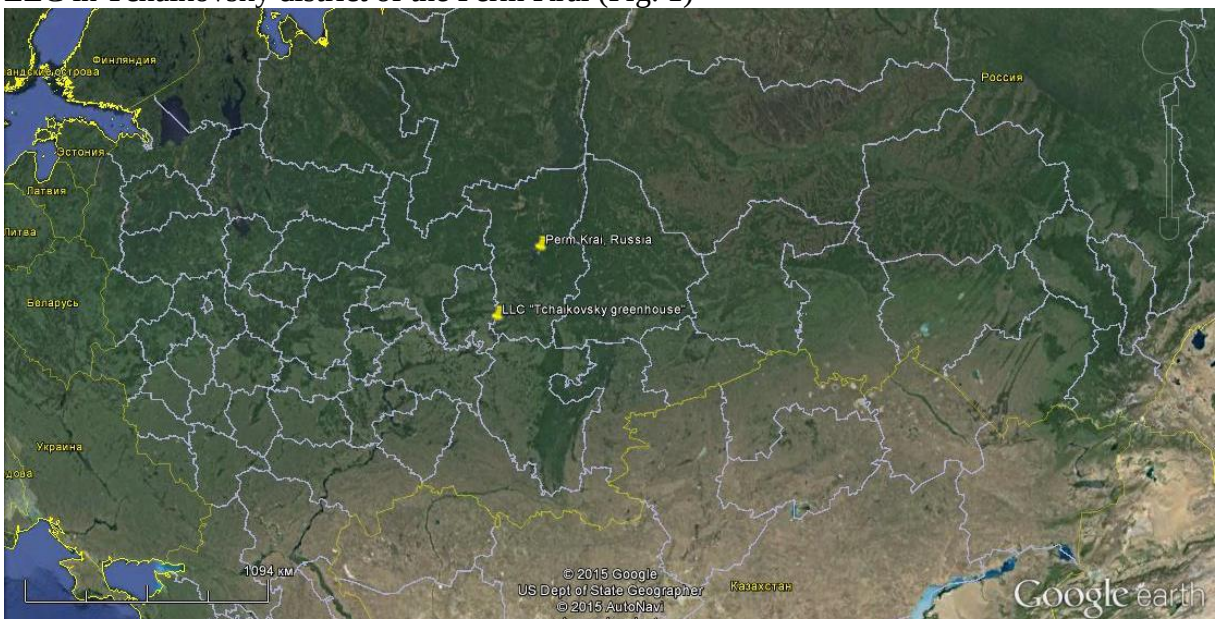

Figure 1. Location of the experiment in Perm, Russia

In the experiment, the following growth regulators were used: Narcissus and Zircon; and the following microfertilizers: Siliplant and Rexolin APN in chelated form. After seeding of lettuce, the substrate was poured with warm water to achieve a moisture content of 65% of full moisture (FM), then with water solutions of study formulations at doses indicated on the label of their packages.

The experiment was using the following scheme:

1. Water (control)
2. Narcissus 0.25 %
3. Zircon 0.01 %
4. Siliplant 0.20 %
5. Rexolin of APN 0.02 %

There was eightfold replication of the experiment variants.

The Geyser lettuce is grown in protected ground year-round. When choosing the lettuce for testing, attention was paid to early ripening, shade tolerance, resistance to bolting and root rot, to the rapid formation of leaf apparatus and healthy appearance of the leaf. Growing season of the lettuce in perfusion culture is about 30 days. Plants were grown in plastic pots (PR-306) with a diameter and a height of 5 cm and perforated bottom. The pots were installed into a cassette. The lettuce seeding was carried out manually on June 7, harvesting on July 14. Before emergence (June 9) cassettes with pots were kept in germination chamber. Then the lettuce was checked and lined out, the number of plants in each pot was set to 5. After 2-4 true leaves appeared, the lettuce plants were placed in special "inserts" cassettes and carried to the work area.

Calcified wood shavings with mineral fertilizers (ammonium nitrate, double superphosphate, potassium sulphate, magnesium sulphate and agrochulk) were used as a substrate: $N\text{-}NO_3$ – 250 mg, P_{water} – 70 mg, K – 350 mg, Mg – 80 mg/l. The substrate was held for 10 days. Agrochemical characteristics of the substrate on the day of seeding are presented in Table 1.

Table 1. Agrochemical characteristics of the substrate

Substratum	EC, mS/cm	Water pH	$N\text{-}NO_3$	K	Ca	P	Mg
			mg/l				
Sawdust on the day of sowing lettuce	0.95	5.60	265.0	302.5	158.0	5.0	90.5

After holding, the substrate had low electrical conductivity of 0.95 mS/cm, which is close to neutral pH 5.60. The content of nitrate nitrogen in the substrate was higher than the required (265 mg), potassium not high enough (302.5 mg), phosphorus very low compared to the required (5.0 mg) and magnesium content slightly above the required (90.5 mg/l).
Lettuce grown in the nutrient solution constantly recycling through the channels and pipes. When preparing the solution, the quality of water was taken into account and readily soluble fertilizers were used: akvarin-12, magnesium sulfate, potassium sulfate, and 50 % nitric acid.
The climatic conditions in the greenhouse have been violated during the experiment. The air temperature in the summer months in 2010 reached 40 °C, and in the greenhouse, especially during the day, it was about 60-70 °C. The ventilation system at the production was not designed for these conditions.

Results and discussion

The yield of green crops in the protected ground is a basis for providing the population with fresh vegetables all year round. It depends on the climatic, biological, agrotechnical, organizational and economic conditions and the properties of the substrate. The average yield of lettuce in the experiment is shown in Table 2.

Table 2. Yields lettuce, depending on the applied growth regulators and microfertilizers, g/ 5 plants

Variant	Average productivity	Increase to control
Water (control)	34.25	-
Narcissus 0.25 %	39.45	+5.20
Zircon 0.01 %	45.05	+10.80
Siliplant 0.2 %	29.10	-5.15
Rexolin of APN 0.02 %	37.85	+3.60
$LSD_{0.95}$	3.09	

The yield of lettuce in the experiment is low and ranges from 29.10 g to 45.05 g from five plants. One of the reasons for low yields is the high air temperature during the growing season of the lettuce. Antipova (2003) points out that the temperature of 18-19 °C is optimal to obtain a high yield of lettuce in greenhouses. Overheating lettuce leaves leads to the closure of stomata, reduces CO_2 intake and reduces photosynthesis. The warm nutrient solution and substrate contributed to increased evaporation of water by plants and non-proportional absorption of ions from the solution, which led to disruption of metabolic processes in plants.
To be sold in the retail network, the lettuce should have 6-7 true leaves and the average weight about 100 g (Antipova, 2010). Growth regulators Narcissus at a dose of 0.25 % and Zircon at a dose of 0.01 % of the solutions had a positive impact on the yield of lettuce, which was 39.45 g and 45.05 g from five plants. The highest yield increase of 10.80 was observed in the Zircon. Apparently at high air temperature Zircon enhanced the enzymatic and hormonal activity creating a more favourable environment for cell division and protecting plants from UV radiation. Peregudov (2010) notes that Zircon is also involved in the regulation of gas exchange and increment of photosynthetic activity affecting the nutrition of plants and yield. When pouring the substrate with 0.25 % aqueous solution of Narcissus a smaller, but still significant increase of 5.20 g was achieved (least significant difference $LSD_{0,95}$ = 3.09 g/5

plants). Shevlyagin (2010) writes that Narcissus penetrates into plant cells and activates an enzyme complex improving the immune system and the work of leaf apparatus. Due to the enhanced plant immunity and metabolism followed by lower severity of pathogens, there is a stable growth of plants and the formation of a high yield.

There was not achieved any effective action of the Siliplant fertilizer on the lettuce, on the contrary, pouring the substrate with it leads to a significant reduction of yield (29.10 g). Siliplant is a mixture of macro and micronutrients: silicon, potassium, iron, magnesium, copper, zinc, manganese, molybdenum, cobalt and boron. The absence of increase with Siliplanta is probably due to lettuce insensibility to silicon, as well as accumulation of a large number of nutrients available to plants at different values of pH of the medium, and their possible antagonistic action. The Rexolin APN microfertilizer contains practically the same micro elements in chelated form. Such nutrients are readily available for the plants. The average yield of lettuce with Rexolin APN is 37.85 g from five plants. There is a significant increase of 3.60 g compared to the control.

The dynamics of mass gain of the lettuce for four control periods shows that the smallest mass was noted in the version with water, which in the last observation period rose sharply to 9.09 g and reached 34.25 g (fig. 2).

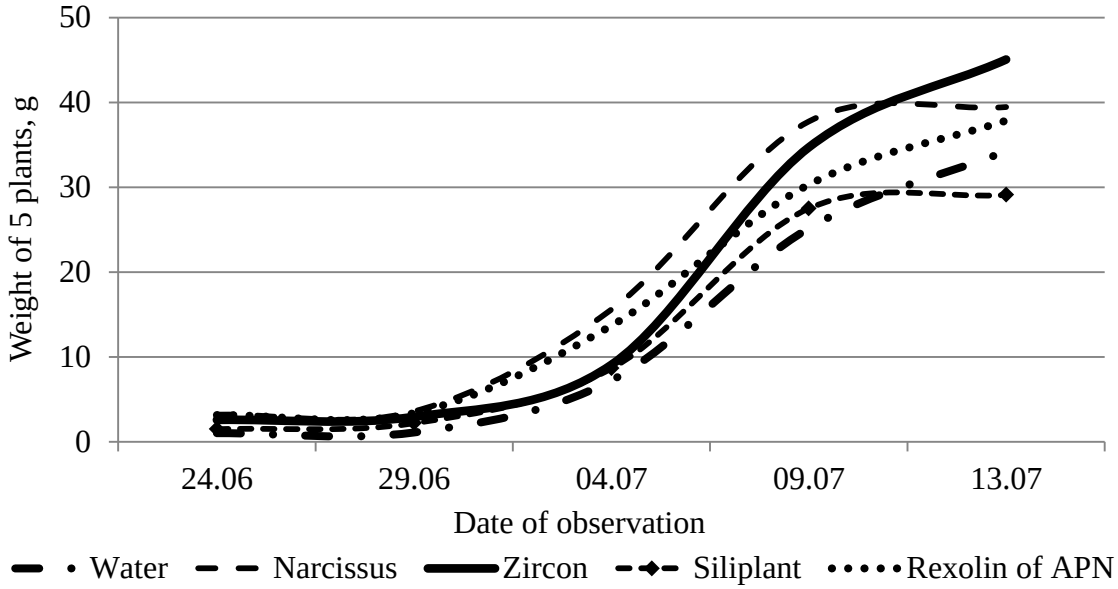

Figure 2. The dynamics of mass gain of the Geyser lettuce depending on applied growth regulators and microfertilizers, g/5 plants

With Zircon mass of plants in the initial period increased slowly, then a sharp increase up to 45.05 appeared. With Siliplant and Narcissus a sharp increase of vegetative mass was noted in the middle of the observations, yet it was then suspended. Slight lettuce mass gain at all observation periods was noted with the use of Siliplant.

Figure 3 shows the dynamics of the growth of lettuce from June 15 to July 13.

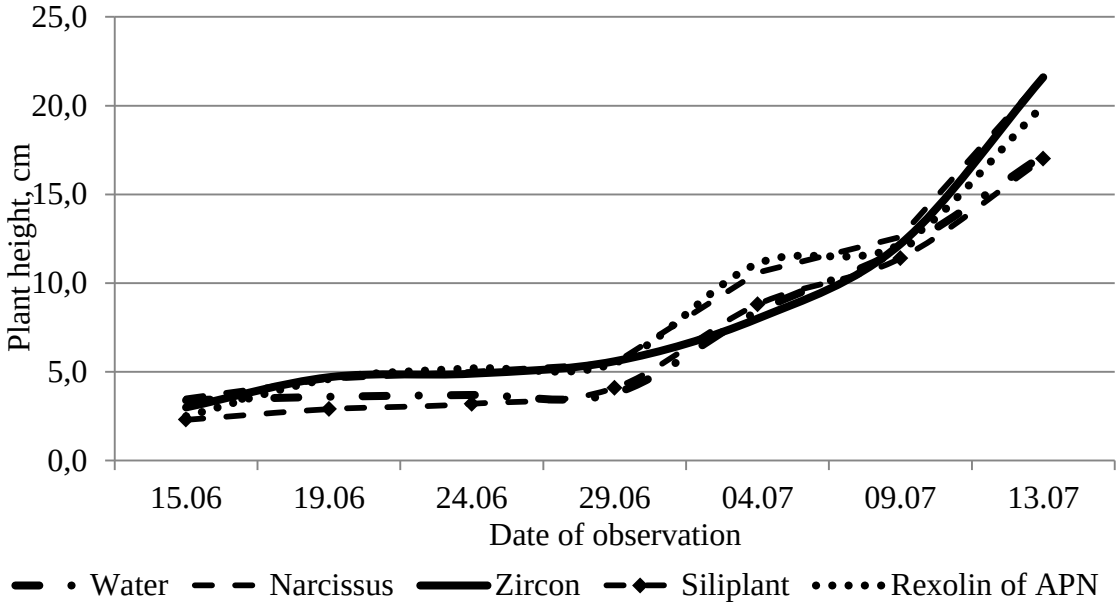

Figure 3. The dynamics of growth of the Geyser lettuce depending on applied growth regulators and microfertilizers, cm

Through the growing season, plants showed growth retardation in the version with Siliplant. Plants using Zircon and Narcissus had the largest increase. However by the end of the growing season all the plants had specified height which according to Antipova (2003) must be 16 to 20 cm average.

Leaf area per plant is shown in Figure 4. The largest area was observed in lettuce leaves with Zircon. By the end of growing season it was 476.25 cm^2. The smallest area of 304.37 cm^2 was observed with Narcissus. Rexolin APN contributed to intensive growth of leaf area during the second half of the growing season and by the end of vegetation the leaf area was 428.75 cm^2.

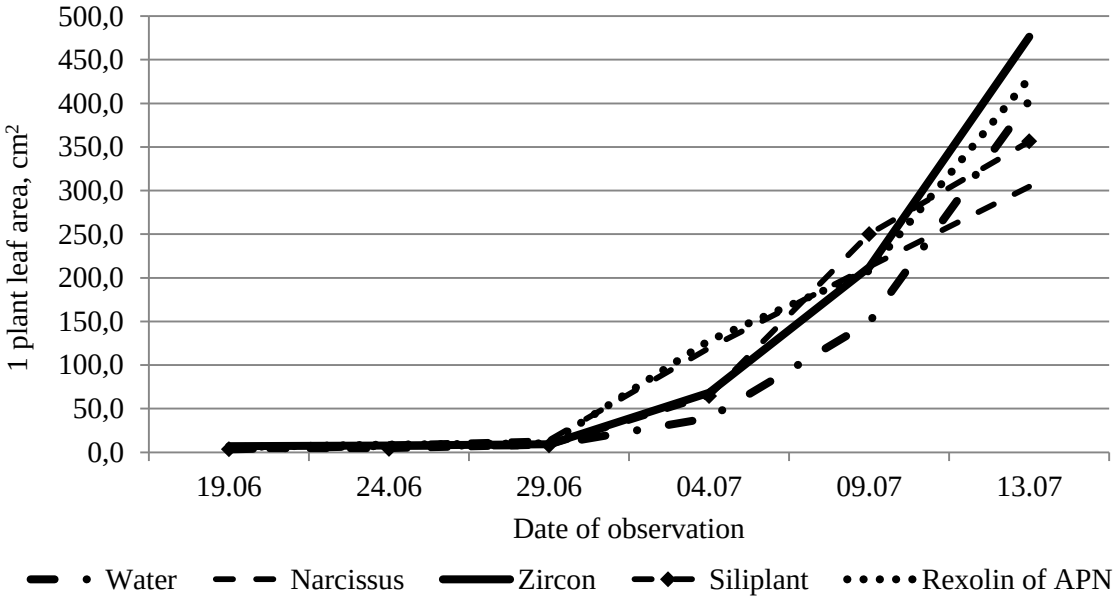

Figure 4. The dynamics of leaf area gain of the Geyser lettuce depending on applied growth regulators and microfertilizers, cm^2

Lettuce as well as other vegetables have a high water content which ranged from 85.4 to 90.0 %. The use of growth regulators contributed to the accumulation of water in plants up to

88.8-90.0 %. In variants with the use of fertilizers a tendency to accumulate dry matter up to 13.2-14.6 % appeared.
The elemental composition of the lettuce is given in Table 3.

Table 3. The elemental composition of lettuce grades Geyser, % on dry matter

Variant	N	P	K	Ca	Mg	Ashes
Water (control)	3.84	0.23	1.86	0.12	1.36	11.65
Narcissus 0.25 %	4.38	0.30	2.07	0.18	1.32	11.90
Zircon 0.01 %	4.81	0.24	2.08	0.24	1.25	11.97
Siliplant 0.20 %	4.57	0.28	2.07	0.23	1.19	11.60
Rexolin of APN 0.02 %	4.58	0.29	2.07	0.31	1.29	11.82
$LSD_{0.99}$	Ffact. < Ftheor.		0.05	Ffact. < Ftheor.		

The total amount of nitrogen compounds in the lettuce is high, ranging from 3.84 to 4.81 %. Growth regulators and microfertilizers had no influence on the content of total nitrogen in plants ($F_{fact.} < F_{theor.}$), however there is a tendency to its increase. Of nitrogen compounds, the lettuce accumulated large amounts of nitrate from 3250 to 4899 mg/kg of crude product, which in all cases exceeded MPC (3000 mg/kg of crude product. Practical Course…, 2001). High levels of nitrates in the lettuce are caused by the use of nitrate fertilizers and high temperature of the nutrient solution. The high plant density resulting in poorer lighting conditions could also contribute to the accumulation of nitrate (Belyaeva, 2008). Puchalskaya (2009) notes that the increase of proportion of potassium in the amount of cations in the solution has a positive effect on nitrate uptake by plants. The highest nitrate concentration in the lettuce was gained in the version with fertilizers: Siliplant – 4899 mg, which 1.63 times exceeds the maximum permissible concentration; Rexolin APN – 3787 mg (1.26 times more than the MPC). The lowest nitrate concentration in the lettuce was gained in the version with growth regulators: Zircon – 3250 mg, Narcissus – 3405 mg/kg (1.08 and 1.13 times, respectively).
The lettuce accumulated ash elements – potassium and magnesium. The potassium content in the lettuce in the variant with water was 1.86 %. Growth regulators, as well as fertilizer equally influenced the content of potassium in the lettuce – 2.07-2.08 % ($LSD_{0.99}$ = 0.05 %). Puchalskaya (2009) in her researches shows that the nitrate nutrition increases the intensity of the potassium absorption by roots of plants and there is a rapid movement of potassium ions with ascending stream. Plants in all variants, along with nitrate nitrogen, had a high content of magnesium of 1.19-1.32 %, which is not connected with the use of growth regulators and fertilizers.
We have calculated the economic removal of nitrogen, phosphorus, potassium, calcium and magnesium with lettuce harvest (Table 4).

Table 4. Carrying out of removal of element of lettuce grades Geyser, mg/ 5 plants

Variant	N	P	K	Ca	Mg
Water (control)	163.2	9.8	79.1	5.1	57.8
Narcissus 0.25 %	193.6	13.3	91.5	8.0	58.3
Zircon 0.01 %	221.3	11.1	95.7	11.0	57.5
Siliplant 0.20 %	194.2	11.9	88.0	9.8	50.6
Rexolin of APN 0.02 %	229.0	14.5	103.5	15.5	64.5

Lettuce characterized by high nitrogen (163.2-229.0 mg), potassium (79.1-88.0 mg), and magnesium (57.8-64.5 mg/5 plants) removal. The value of the elements removal from the substrate is associated with lettuce dry matter yield and concentration of elements in it.

Conclusion

On the basis of conducted studies we can conclude the following:

1. Siliplant can't be recommended for growing lettuce in greenhouses because formed a low level of productivity with a high content of nitrates.

2.When growing leaf lettuce in greenhouses can recommend Pouring of substrate with 0.01% Zircon solution, 0.25 % Narcissus solution, or 0.02 % solution of Rexolin APN microfertilizer.

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THE EFFECT OF WEEDS ON THE YIELD AND QUALITY PARAMETERS OF RAPESEED

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Abstract

It has been shown that the presence of weeds can signifficantly affect the yield of winter rapeseed, especially during years with high humidity. The weed species *Sinapis arvensis*, *Raphanus raphanistrum*, *Stellaria media*, *Solanum nigrum* and *Chenopodium hybridum* are particularly harmful because of their fast development. The study was carried out in 2011/12 (Crvenka, Novi Sad) and 2013/14 (Kovilj, Novi Sad). 14 herbicides and their combinations were applied, to assess the possibility of controlling the weeds in the rapeseed plots and the impact of weed control on the amount of proteins and oils.

The active substance and their combinations: metazachlor and clomazone showed the highest efficacy on annual broadleaf weeds, except on *Sinapis arvensis*, *Solanum nigrum* and *Chenopodium hybridum*. The differences in the quality and quantity parameters of rapeseed, although observed, were not statistically signifficant. The spring application of active substance metazachlor did not show fitotoxicity effects on rapeseed plants.

A higher percentage of oils in the rapeseed was determined at the site Novi Sad in 2011/12, when compared to the site Crvenka. Data analysis of the oil and protein contents in the studied sites in 2013/14 has shown no signifficant differences between the sites.

Therefore, it can be concluded that the observed differences in the oil and protein contents are the result of the effects of climatic factors, the presence of weed species, on which the herbicides weren't efficient enough, edaphic factors, etc.

Keywords: *weeds*, *herbicides*, *rapeseed*, *oil content*, *protein content*

Introduction

Brassica napus, commonly known as rapeseed, is the dominant biofuel crop in the European Union (EU) (van der Velde et al., 2009), with its economic significance increasing worldwide (Kachel-Jakubowska and Szpryngiel, 2008). It is the fourth most important crop by area in the EU, after wheat, maize and barley (Ollier & Utz, 2007), its main producing countries being France, Germany, Poland and the Czech Republic (van der Velde et al., 2009). Further increases in the area covered by rapeseed in the EU are to be expected in the near future (Ollier & Utz, 2007). Rapeseed was sown in Serbia on 6273 ha in year 2000, 13 965 ha in 2006 (with 13133 ha in Vojvodina province), 15000 ha in 2010 and 9686 ha in 2013 (Stat. Yearb. Serb, 2014). The median production of rapeseed for France is estimated to be 22057 t and for Italy 62500 t (FAO, 2013), while for Serbia it is 26992 t (2,8 t/ha) (Stat. Yearb. Serb, 2014). Traditionally, rapeseed has been grown primarily for food, e.g. vegetable oil (40-45%), and for feeding purposes, as a high-protein (25%) animal feed (van der Velde et al., 2009, Roshdy, et al., 2008).

There is ample evidence of the harmful effect of weeds on crop plants, as a result of the competition for light, water, nutrients and space, or as a result of allelopathic interactions between weed species and the crop plants (Rose and Bell, 1982; Roshdy et al., 2008). Many weeds can, in addition to direct damage, also cause indirect damage as vectors of harmful fungi and insects, e.g. weed species *Capsella bursa pastoris* is host to a fungus *Albugo*

candida (Leino, 2006; Antonijević and Mitrović, 2007). Furthermore, the presence of weed seeds in the harvested rapeseed crops can reduce the total yield of oils and proteins (Klaaßen, 2006; Davies, 2005).

Rapeseed, as a slow-growing plant, is particularly vulnerable to the severe competition of weeds. The faster development of weed plants causes shading of the rapeseed, thereby reducing its photosynthetic efficiency and subsequently lowering the final yield and crop quality. Furthermore, weeds with vigorous, highly branched root systems can also deprive the rapeseed of the necessary soil nutrients, thereby inhibiting its development (Roshdy et al., 2008). Longterm agricultural practice has shown that *Sinapis arvensis*, *Raphanus raphanistrum, Stellaria media, Solanum nigrum* and *Chenopodium hybridum* are particularly harmful to rapeseed yield and quality due to their fast development (pers. obs.).

It is important to assess the efficiency of weed control on the reduction of losses in the yield and quality parameters of rapeseed. Therefore, the purpose of this paper is to present the results of an initial study of the effects of herbicide weed control on the yield and quality parameters of rapeseed. The results are based on the yield samples taken from the infested rapeseed fields in two study sites, with the aim to assess the effect of these weed species on the rapeseed yield and quality parameters.

Materials and methods

Weed control in spring rapeseed was carried out in 2011/2012 (loc. Novi Sad and Crvenka) and 2013/2014 (loc. N. Sad and Kovilj), using the standard method for testing the efficiency of herbicides in rapeseed crops (Anonymus, 2007; Table 1). The experiment was set up as a randomized block design with three replicates. Applied herbicides were based on active substances metazachlor and clomazone. Samples for assessment were taken from each treatment (3 times) and compared using the *t-test*. Rapeseed yield and quality were determined by measuring and analyzing the following parameters: grain yield (kg/plot ($30m^2$)), oil content (%) and protein content (%) in the seeds.

Table 1. Basic data for the experiment

Location	Crvenka	Novi Sad	Kovilj	Novi Sad
Soil type	chernozem	Degraded chernozem	chernozem	Degraded chernozem
Previous crop	Wheat	Seed pea	Wheat	Seed pea
Planting date	09.09.2011.	07.09.2011.	08.09.2013.	05.09.2013.
Application date	10.09.2011 preem	09.09.2011 preem	11.09.2013 preem	08.09.2013 preem
	10.03.2012. postem	10.03.2012. postem	05.03.2014. postem	05.03.2014. postem
Assessment date	1st assessment: 12.10.2011. 2nd assessment 18.03.2012.	1st assessment: 11.10.2011. 2nd assessment 18.03.2012.	1st assessment: 14.10.2013. 2nd assessment: 10.03.2014.	1st assessment: 14.10.2013. 2nd assessment: 10.03.2014.

Results and discussion

Observed results have shown that the changes in the oil and protein content (depending on the herbicides applied and the presence of weed species) did not significantly affect the rapeseed yield in all of the tested sites (Table 2a).

Also, no effect of climate factors on the rapeseed yield was evident in the locality Novi Sad, when observed in two consecutive growing seasons (results not shown). Peltonen et al. (2008) have concluded that in their experiment the changes observed in the yield were linked to the yield-associated changes in the rapeseed quality. Furthermore, oil yields increased due to both higher oil content and higher yields, while increases in protein yield only resulted from higher seed yields (Peltonen et al., 2008).

Table 2a. Rapeseed yield based on the oil and protein content

locality	year	T value		P value		St.dev	
		oil	protein	oil	protein	oil	protein
N. Sad	2011/12	-80,63	-108,65	0,000	0,000	0,83	0,97
Crvenka	2011/12	-17,38	-18,27	0,000	0,000	0,72	0,62
Kovilj	2013/14	-80,21	-84,38	0,000	0,000	1,22	0,57
N.Sad	2013/14	-103,49	-85,76	0,000	0,000	1,002	1,01

p<0.05*, p<0.01**, p<0.001***

The analysis of the weed species presence and abundance has indicated that the applied herbicides have shown low efficacy on *Raphanus raphanistrum, Stellaria media, Solanum nigrum* and *Chenopodium hybridum*. Very low efficacy was detected for the species *Sinapis arvensis* (loc. Crvenka). Regrowth of plants (10-15%) in the spring has caused the yield reduction (with statistical signifficance, Table 2b). In the locality N. Sad low efficacy was observed on the species *Solanum nigrum* and *Chenopodium hybridum.* Similar conclusions were made by Rose and Bell (1982), who pointed out that growth of some of the weed species such as *Sinapis arvensis* and *Thlaspi arvensis* in rapeseed fields reduced the *B. napus* seed quality, by increasing the level of erucic acid in the extracted oils and the glucosinolates content of the remaining meal. The application of herbicides in the spring, especially those based on the a.s. metazachlor, did not cause the reduction in the grain yield, and oil and protein content. Our results of different methods of weed control testing: 1) chemical and 2) non-chemical method of eradicating weeds by manual hoeing, concur with those obtained by Tanveer et al. (2005). They have concluded that manual hoeing as a method of weed control is not feasible and has become too expensive due to the costs of manual labor. Bearing that in mind, chemical weed control is the best alternative, being less laborious, time saving and more effective.

Table 2b. Comparison of rapeseed yealds from different localities

locality	year	means	T value	P value
Crvenka	2011/12	411.33	-2.7	0.01
Novi Sad		470.33		
Crvenka	2011/12	411.33	-4.14	0.0003
Kovilj		499.33		
Crvenka	2011/12	411.33	-3.6	0.001
Novi Sad	2013/14	489.00		
Kovilj	2011/12	499.33	+ 4.1	0.0003
Novi Sad	2011/12	470.33		
Kovilj	2011/12	499.33	1.7	**0.1 NS**
Novi Sad	2013/14	489.00		
Novi Sad	2011/12	470.33	-2.5	**0.02***
Novi Sad	2013/14	489.00		

p<0.05*, p<0.01**, p<0.001***

Using the t-test and obtained data from all locations, there was no statistical significance in the differences between the tested localities, based on the oil content (with the exception of loc. N.Sad, p=0,014, Table 3), and proteins (with the exception of loc. N.Sad and Kovilj, p=0,039, Table 4). Contrary to this, the results of RajabLarijani and Alikhani (2011) indicate that rapeseed yield, yield components and oil percentage decreased significantly due to weed interference.

Table 3. Oil content changes in rapeseed plants

locality	year	Means			T value			P value		
		I	II	III	I	II	III	I	II	III
N. Sad	2011/12	45,90	45,20	45,46	16,96	9,55	8,32	0,000	0,000	0,000
Crvenka		41,08	40,25	42,35						
N. Sad	2013/14	45,90	45,23	45,46	4,81	5,25	8,07	0,000	0,000	0,000
Kovilj		44,06	43,75	42,35						
N. Sad	2011/12	45,90	45,22	45,46	5,42	3,52	2,61	0,000	0,000	**0,014**
N. Sad	2013/14	44,09	43,56	44,22						

p<0.05*, p<0.01**, p<0.001***

Our results (Tables 3, 4) can be explained by the conclusions of Peltonen et al. (2008). Their findings indicate that relatively low variations in seed oil content are strongly determined by the oilseed genotype. This is further supported by the findings that the seed number per square meter also does not correlate with the seed oil content, while the variation in single seed weight is quite similar to that in oil content (Peltonen et al., 2008).

Table 4. Protein content changes in rapeseed plants

locality	year	Means			T value			P value		
		I	II	III	I	II	III	I	II	III
N. Sad	2011/12	17,94	17,56	18,29	-14,43	-5,56	-4,19	0,000	0,000	0,000
Crvenka		22,38	20.56	19,93						
N. Sad	2013/14	21,98	22,00	21,22	4,02	4,25	-2,16	0,000	0,000	**0,039***
Kovilj		20,83	21,45	22,00						
N. Sad	2011/12	17,94	17,33	18,29	-11,18	-9,35	-7,16	0,000	0,000	0,000
N. Sad	2013/14	22,00	21,33	21,21						

p<0.05*, p<0.01**, p<0.001***

The hectoliter weight of seed ranged from 69,0 to 70,2 g in the loc. of Kovilj, from 67,5 to 68,8 g (in 2013/14) and from 67,4 to 69,0 g (in 2011/12) in the loc. N. Sad and from 67,6 to 69,0 g in the loc. Crvenka. In our study, the 1000 seeds weight in all of the localities was between 4,27 (recorded in the weedy check) and 5,40 g, while Khan et al. (2003) have previously documented a stronger effect of weeds on the 1000 seeds weight - 2,27 g.

Conclusions

The results of this research have shown that the changes in the oil and protein content did not affect the yield of the rapeseed. Also there were no statistically significant differences observed in the oil and protein contents in the seeds between locations. Furthermore, the application of the tested herbicides in the early spring has not caused a reduction in either the grain yield or the oil and protein content of the rapeseed, hence it can be said that no effects on yield were registered due to the good effects of tested herbicides.

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MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF FUSARIUM SUBGLUTINANS, PATHOGEN OF ANISE SEED IN SERBIA

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Abstract

During 2012 and 2013 aniseeds were collected in three localities in the province of Vojvodina (Mošorin, Veliki Radinci and Ostojićevo). Conventional mycological seed testing revealed significant seed infection with *Fusarium* spp. ranging from 2-18%. From the infected anise seed, monosporial cultures, based on morphology, were identified as *Fusarium subglutinans*, and their pathogenicity proved on artificially inoculated anise seedlings. Molecular identificaton was performed utilizing polimerase chain reaction (PCR) with primer pair EF1/EF2 and by amplification of the translation elongation factor-1 alpha (*tef-1α*) gene. The sequence analysis of *tef-1α* region revealed that Serbian isolate A5 (GenBank Accession No. KP126606) shared 100% nt identity of with sequences of seven *F. subglutinans* isolates deposited in the GenBank. The presence of a large number of phytopathogenic fungi on anise seed should be further investigated in order to clarify their relationships and relative significance.

Key words: anise seed, *Fusarium subglutinans*, molecular identification, morphological features, pathogenicity test

Introduction

Anise (*Pimpinella anisum* L.) is an annual important spice and medicinal plant belonging to the family *Apiaceae*. Aniseed is cultivated in Turkey, Asia, South Africa, Europe and America. The anis fruit oil is used in the pharmaceutical industry to mask odors and for flavoring. The aniseed has economical importance as a flavoring agent also in food and perfumery industries (Baser, 2002; Tabanca et al. 2007). Since anise is an attractive ornamental and medicinal species, with seeds rich in essential oils, its production in Serbia has increased over the last decade. Recently, an increasing interest in the cultivation and production of medicinal and aromatic plants has been noticed (Mahmoud, 1996), but the farmers are facing a significant low productivity and low content of active ingredients in these plants because of fungal diseases and also the synthetic chemicals which are employed as fungicides in crop protection (Fiori et al. 2000; Hashem et al. 2010).

The genus *Fusarium* is widely found in plant debris and crop plants worldwide (Marasas et al. 1984). Several species from this genus are economically relevant because, apart from their ability to infect and cause tissue destruction on important crops such as corn, wheat and other small grains on the field, they produce mycotoxins on the crops in the field and in storage grains (Dambolena et al. 2010; Whang et al. 2011). Diseases that are reported on anise are being intensivly studied in many countries worldwide, while in Serbia published literature data about anise pathogens are scarse although disesases cause major problems to anise production. *Fusarium* spp. were common pathogens on anise seed during 2012 and 2013 at the cultivating plantation of the Institute for Medicinal Plants Research “Dr Josif Pančić”, Belgrade (Serbia).

Consequently, the objectives of this study were to identify the *Fusarium* species of anise seeds for the first time in Serbia based on morphological and molecular characteristics.

Material and methods

Identification of *Fusarium* species

During 2012 and 2013 aniseeds were collected in three localities in the province of Vojvodina (Mošorin, Veliki Radinci and Ostojićevo). A hundred aniseeds were randomly chosen from every sample in 4 replications. For agar plate method, the aniseeds after surface sterilization with 2% NaOCl for 2 min, were rinsed twice in sterile distilled water and dried on sterile filter paper in a laminar flow cabinet. Aniseeds from each sample were placed on PDA and the Petri dishes were incubated for 7 days at 25°C with a 12 h dark ⁄ light cycle (Singh et al. 1991). The fungal species present on each seed were recorded and the percentage incidence of each fungus per sample was computed.
The morphology of macroconidia, microconidia, conidiogenous cells and the chlamydospores was assessed from cultures grown on Spezieller Nahrstoffarmer Agar (SNA) and carnation leaf agar (CLA). Colony color and colony diameter were recorded after 7 days of growth on PDA (Burgess et al. 1994). Morphological identifications of isolates were made using the criteria of Gerlach and Nirenberg (1982) and Leslie and Summerell (2006).

Pathogenicity

Pathogenicity tests were conducted on five healthy, 1-month-old seedlings of anise in a moist chamber. Plants were separately inoculated in pots with sterilized soil and 10 ml of *Fusarium* sp. isolate spore suspension (1×10^6 conidia/ml). Control plants were inoculated with sterile soil without spore suspension. After inoculation, the plants were placed in a humidity chamber for 24h and then transferred to a greenhouse maintained at 27°C. The development of symptoms on inoculated and control seedlings were observed for 10 days. The inoculated fungi were re-isolated from the infected plants to prove the Koch's postulates.

PCR detection and sequencing

Total DNA was extracted from fungal mycelium with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. PCR was carried out for the identification of *Fusarium* species by amplifying the translation elongation factor-1α gene (*tef-1α*) using the primer pairs EF1 and EF2 as described in O'Donnell et al. (1998). PCR was performed in a 25 µl l final reaction mixture containing 12.5 µl 2 X PCR Master mix (K071, Fermentas, Lithuania), 9 µl RNase-free water, 1.25 µl each of both forward and reverse primers (100 pmol/µl, Metabion International, Deutschland) and 1 µl template DNA. DNA amplification was performed in a Thermal Cycler PCR system 2720 (Applied Biosystems).
Amplified products were analyzed by 1% agarose gel electrophoresis, stained with Midori Green DNA Stain (Nippon Genetics), and visualized under a UV transilluminator. Sequencing in both directions was performed on an automated sequencer (ABI 3730XL Automatic Sequencer Macrogen, Korea). Sequence generated in this study was deposited in the National Center of Biotechnology Information (NCBI) GenBank database. Sequence of Serbian representative isolate (A5) was compared with sequences of *Fusarium* species available in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) using the ClustalW program (Thompson et al. 1994) and MEGA5 software (Tamura et al. 2011).

Results and discussion

Conventional mycological seed testing revealed significant seed infection with *Fusarium* spp. ranging from 2-18%. In this study, a total of eight *Fusarium* isolates were obtained from aniseeds, collected in three different localities in the province of Vojvodina. Based on their morphological characteristics, these isolates were identified as *F. subglutinans*. After 7 days of incubation at 25°C under 12 h of dark ⁄ light cycle, eight monoconidial isolates formed colonies with white aerial mycelium, purple pigment in agar and orange sporodochia on the surface of PDA (Fig.1a,b). Within the colony, microconidia were hyaline, non-septate and ovoid, and ranged from 12.5 to 16.5 × 3 to 3.5 µm (Fig.1d). On CLA colonies produced thin-walled macroconidia with 3 to 5 septa, a foot-shaped basal cell and a curved apical cell (33-51 x 2.5-3.6 µm from 100 measured) (Fig.1c). The morphological characteristics of the pathogen correspond with the records for *F. subglutinans* described by Leslie and Summerell (2006).

A total of eight isolates of *F. subglutinans* obtained from naturally infected seedlings of anise were tested for pathogenicity. Within 10 days, inoculated plants developed leaf wilt, stunted growth, discolored vascular tissue on stems, and finally died. No symptoms were observed on any of the control seedlings.

The amplification of *tef-1α* produced a sequence of 629 bp (Fig. 2) for *F. subglutinans*. The sequence analysis of *tef-1α* region revealed that the representative Serbian isolate A5 (GenBank Accession No. KP126606) shared 100% nt identity of with seven *F. subglutinans* isolates deposited in the GenBank from China (JX456582 to 83, KC964122 to 23 and JQ639211), and USA (JF270302 and JF270288).

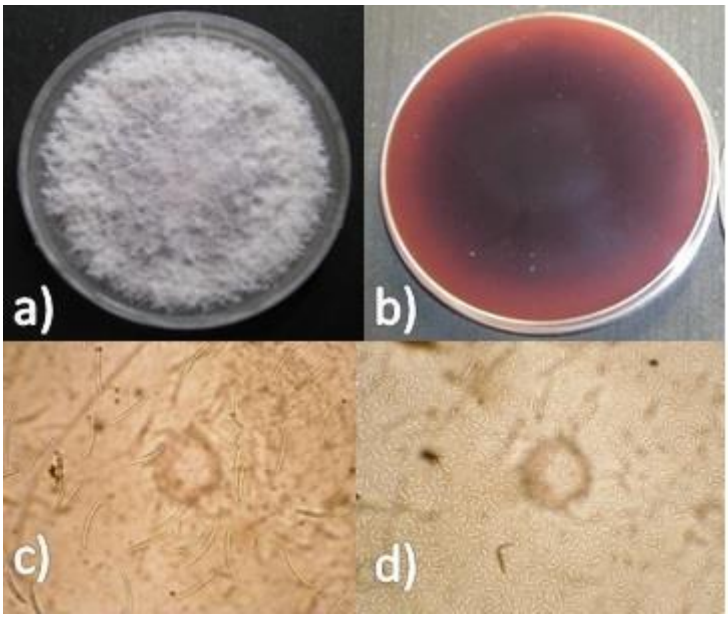

Fig. 1. *F. subglutinans:* a) surface side after 10 days of grown on PDA. b) reverse side. c) macroconidia. d) microconidia

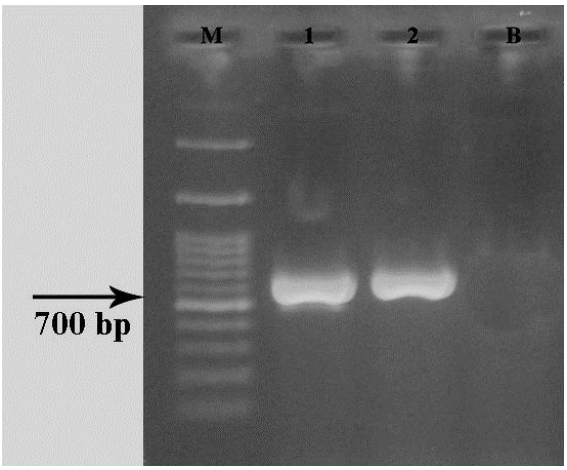

Fig. 2. Electrophoretic analysis of PCR products obtained using primer pair EF1/EF2. Lanes: M - Mass-Ruler^{TM}DNA ladder, Mix (Fermentas Life Sciences GmgH, Lithuania); 1 - isolate A5; 2 - positive control (*F. proliferatum*); 3 - negative control (PCR mix with Rnase-free water)

This study represents the first report which identifies pathogens of *F. subglutinans* in anise seeds in Serbia. This pathogen identification was based on the morphological pathogenicity and molecular identification. Because *F. subglutinans* can cause further losses through the contamination of crops with mycotoxins, these infected seeds are of poor quality reducing the market value of the produce. The obtained results are of great importance as they support the reliable and quick protocol for molecular identification of this pathogen, as a prerequisite for implementing efficient control measures. Previous studies of mycopopulation of anise seeds showed only *Bipolaris sorokiniana* (Pavlović et al. unpublished data) and *Alternaria alternata* presence (Stanojković-Sebić et al. 2014) in Serbia.

Conclusions

From the results obtained in this study, based on the cultural, macroscopic, microscopic and molecular characteristics, selected isolates from anise seeds were identified as *F. sublutinans*. The representative isolates A5 showed the highest nt homology of 100% with seven *F. subglutinans* isolates from China and USA. To our knowledge, this is the first report of natural infection of anise seeds by *F. sublutinans* in Serbia.

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REACTION OF SUGAR BEET GENOTYPES ON CHARCOAL ROOT ROT CAUSED BY MACROPHOMINA PHASEOLINA

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Abstract

Charcoal root rot, caused by *Macrophomina phaseolina* is potentially the most destructive sugar beet pathogen in Serbia, especially during extremely warm and dry season. Reaction of eleven sugar beet genotypes (G1-G11) was tested to *M. phaseolina* using artificial inoculations in controlled, laboratory conditions. The aim of this research was to optimize methods of testing pathogenicity of *M. phaseolina* on sugar beet. Development of such methods would enable testing a large number of commercial genotypes which are grown in Serbia in order to improve control measures against this pathogen, but also to test different sugar beet germplasms for the purpose of breeding for resistance. Three week old plants were inoculated with *M. phaseolina* grown on sorghum seed. Root rot intensity was evaluated after seven days using scale from 0 (healthy plant) to 4 (complete necrosis of hypocotil and root). Results of this research showed that sugar beet genotypes reacted significantly different to this pathogen – root rot intensity in average varied from 1.5 to 3.3. These results showed that more resistant sugar beets can be recommended for planting in areas with high density of *M. phaseolina* inoculum in soil.

Key words: *Macrophomina phaseolina, charcoal root rot, sugar beet, pathogenicity, resistance*

Introduction

Charcoal root rot, caused by *Macrophomina phaseolina* is economically the most important and the most damaging sugar beet root disease in Serbia. Infections are favored by dry and warm conditions, especially those in which sugar beet are grown without irrigation. The disease is most evident at the end of the season, although the fungus can be isolated from beet roots through the growing season (unpublished data). Symptoms are similar as reported on soybean and most apparent under conditions that reduce plant vitality (Kendig et al., 2000), such as high soil temperature, drought, reduced fertility, inadequate cultivation practices, poor soil structure and root injuries from root pests, such as sugar beet root aphids (*Pemphigus fuscicornis*) and other (Stojšin et al., 2012a).

Primarily wilt and subsequently leaf decay occur on individual plants. Later, severely affected roots become rotten while leaves are completely dry. Internally, roots are spongy and dehydrated whereas the color of the inner root tissue can vary from lemon yellow, light or dark brown to black, depending on sugar beet variety, environmental conditions and soil moisture (Stojšin et al., 2012b) – Figure 1.

Figure 1. Symptoms of charcoal root rot on sugar beet root

The parasite survives in plant remains in the field as mycelia and microsclerotia-overwintering structures. Microsclerotia may survive in soil for many years and they are always present in soil in sufficient amount. Infection may occur on young roots, after which parasite spreads through vascular system, while characteristic symptoms appear after plants are being stressed due to drought (Stojšin et al., 1999).

Together with climate changes, which will eventually lead to warmer and drier parts of vegetative season, it is expected that in future charcoal root rot becomes even more significant problem in sugar beet production. Given that *M. phaseolina* is very virulent and polyphagous, the most effective control measure would be use of disease resistant cultivars (Stojšin et al., 2012a).

Therefore, the aim of this research was to test a reaction of 11 sugar beet cultivars and germplasms to inoculations with *Macrophomina phaseolina*. It is expected that results of this research contribute to optimization of pathogenicity tests which will be used for detection of resistance genes in sugar beet cultivars.

Material and methods

Isolate ŠR 62/4 which was used for artificial inoculations was isolated from sugar beet root with severe symptoms of charcoal root rot in 2009. Sugar beet at the locality of isolate's origin suffered great damages during 2009 (locality Ečka, Vojvodina province, Serbia). Inoculum was prepared by colonizing sorghum seed (Omar et al., 2007).

Eleven sugar beet cultivars (G1-G11) were tested. Plants at the two leaf stage were replanted in 500cm³ pots in homogenous containing mixture of sterile sand and inoculum in volume a ratio 3:1 ratio (v/v) (sand:sorghum seed). For the negative control, plants were grown in non inoculated sterile sand. Plants were incubated in a growth chamber at 30°C with a photoperiod of 16h/8h light/dark and watered daily. Symptom development on leaves was assessed daily, while final evaluation of root and hypocotyl necrosis was performed after 7 days, when over 80% of leaves of most susceptible cultivar showed symptoms of irreversible wilting.

Root and hypocotyl necrosis was rated using a scale from 0 to 4 which is explained in Table 1 and Figure 2. Based on average pathogenicity isolates were grouped into three groups: i) low pathogenic 0-1, ii) moderately pathogenic 1-2, iii) highly pathogenic 2-4.

Table 1. Scale for evaluation of symptom development on hypocotil and root.

Grade	Symptom
0	Healthy plant
1	Individual lesions on hypocotyl and root
2	Merging of lesions on hypocotyl and root
3	Section necrosis on hypocotyl and root
4	Complete necrosis of hypocotyls and root

Figure 2. Scale for disease intensity evaluation on sugar beet hypocotil and root (from right to left: 0-healthy plant, 4- complete necrosis of hypocotyls and root).

In order to fulfill Koch's postulates, reisolation of *M. phaseolina* from inoculated plants was performed on standard PDA (Potato Dextrose Agar) with addition of 50µg/ml of streptomycin sulphate. Reisolations were done from 10 plants rated 2 and 3 per cultivar.
Statistical analysis was performed in Statistica 12 software (StatSoft, Tulsa, OK) using ANOVA and Fisher LSD test at p=0.05.

Results and discussion

Seven days after inoculation, 83% of leaves of sugar beet cultivar G4 irreversibly wilted or decayed (Chart 1). At that moment, all plants were pulled out and intensity of charcoal rot on hypocotils and roots was evaluated. Disease severity on root and hypocotil of 11 sugar beet genotypes are shown in Chart 2.

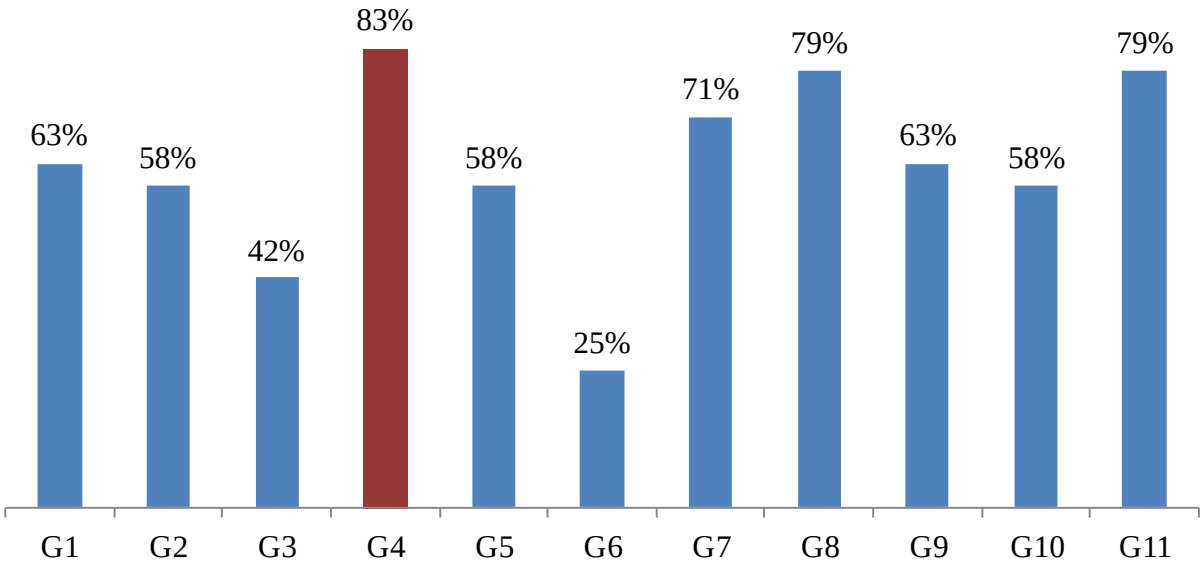

Chart 1. Decay and wilting registered seven days after inoculation (%)

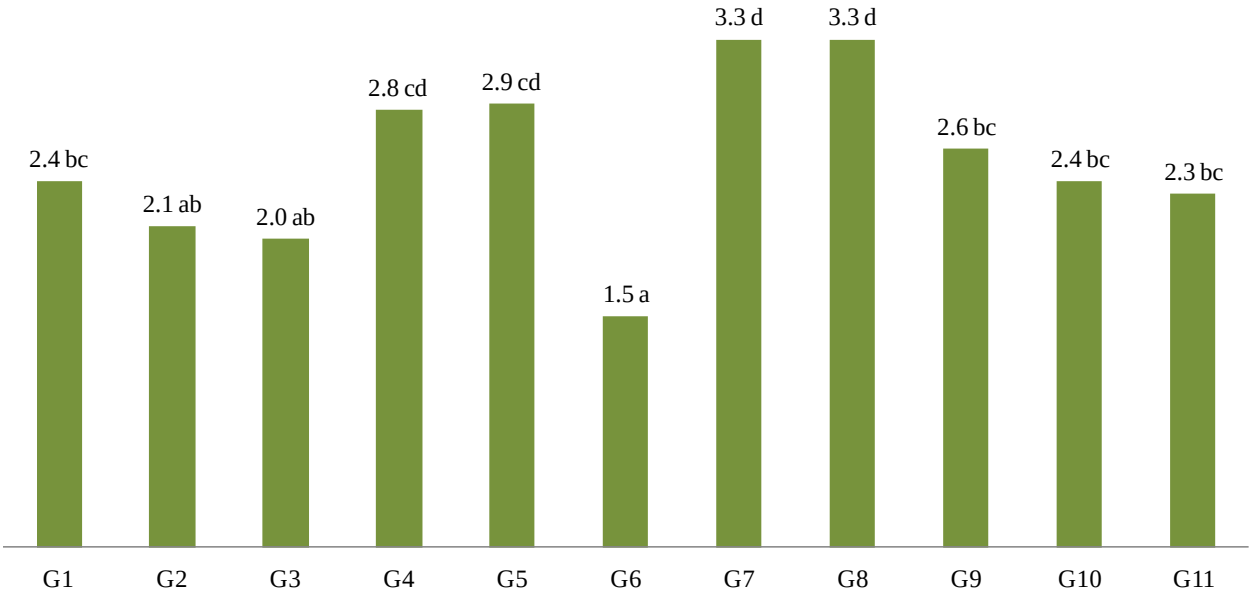

Chart 2. Disease intensity on hypocotils and roots of 11 sugar beet genotypes. Values not connected by the same letter are significantly different at $p<0.05$.

According to leaf ratings, G4 reached 83%, whereas G8 and G11 showed 79% of irreversible wilting and decay 7 days after inoculation (Chart 1). Symptoms on roots and hypocotil corresponded to wilting and decay in case of cultivars G4 (2.8) and G8 (3.3), which belonged to the group of the most susceptible cultivars (Chart 2). As most resistant to charcoal root rot was G6, which showed slowest leaf decay after 7 days (25%) and lowest intensity of root and hypocotil necrosis (1.5).

All cultivars in non-inoculated control were healthy, no wilting or decay was noted and hypocotil and roots were rated 0 (Figure 3).

In order to fulfill Koch’s postulates, reisolation of fungus from infected root with typical simptoms was performed. Results showed that *Macrophomina phaseolina* was isolated in 100% of cases.

Figure 3. Non-inoculated plants (upper row) and inoculated G4 (lower row).

Macrophomina phaseolina is a soil-borne plant pathogen which causes charcoal root rot in different crops including sugar beet. Variations in morphological, cultural, pathogenic and genetic characteristics among isolates are present and well investigated (Sharma et al., 2003; Purkayastha et al., 2006; Iqbal and Mukhtar, 2014). However, the main challenges considering this parasite are means of control. Different measures were described and tested including biological control using *Bacillus subtilis* (Singh et al., 2008), *Trichoderma* species (Ramezani, 2008), fluorescent *Pseudomonas* and *Bacillus* strains (Pal et al., 2001), which all showed potent in control of *M. phaseolina*. Fungicide applications were also tested (Cohen et al., 2012), however, their utilization and cost effectiveness in sugar beet production in Serbia are yet to be investigated. According to Stojšin et al. (2012a), since sugar beet in Serbia is mostly produced without irrigation, environmental conditions have primary significance in disease incidence. Therefore, the most suitable disease control would be breeding sugar beet for resistance similarly as in common bean (Hernández-Delgado et al., 2009) and soybean (Smith and Carvil, 1997).

Conclusions

Significant differences in response of cultivars to infection with *Macrophomina phaseolina* exist. This implies that genes that have an effect on susceptibility of plants during infections with this pathogen could be targeted in order to breed sugar beet for resistance to charcoal root rot. This method should be used for further testing of larger number of cultivars with different response to infections with *Macrophomina phaseolina* in the field.

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THE SISTEM OF REGULAR TEHNICAL DEVICE CONTROL FOR THE APPLICATION OF PESTICIDES IN THE REPUBLIC OF SERBIA

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Abstract

The production of healthy food and preserving the environment is a fundamental objective of modern agricultural production. Proper chemical protection is important for preventive crop protection against diseases, pesticides control, protection against disease and controlling weeds. In Serbia in the category "attachable unit" there was a total of 138.084 sprayers and 17.281 atomizers. Percentage of sprayers more of ten years old in the Republic of Serbia is 82%. This data points out to the fact that protection, as well as agro-technical measures, in Serbia are performed with the unreliable devices that may seriously undermine production. Plant protection department of the Ministry of agriculture and environment of the Republic of Serbia in accordance with the Directive of the European Parliament 2009/128 EC establishes a framework for the control systems of sprayers and atomizers. According to the standards of the European Union (EU) is necessary to optimize the consumption of pesticides and reduce their harmful impact on the environment and human health. Testing of working machines is in accordance with the European norms EN 13790 which prescribes methods and equipment used to perform inspections. This allows agricultural producers to export their products to the EU market which need to fulfill all the conditions prescribed by the "Global G.A.P." standard. This directive becomes current in our country, so that the adoption of relevant legislation in this field is expected in the future, together with Serbian joining in EU.

Key words: *technical control, pesticides, sprayers, atomizers*

Introduction

The main task of modern agriculture is the production of safe food. Regular and agro-technical measures inevitable in the process of agricultural production is the care and protection of crops from diseases, pests and weeds. The chemical method of protection is now the primary means of plant protection. Pesticides are intended to prevent, destroy or control pests, but their remains in food, water, soil and animal feed have resulted in poisoning of wildlife and domestic livestock, environmental pollution and disruption of ecological balance (Bugarin et al., 2010; Srivastava, 2014).

Using the correct techniques and controlled application of pesticides, in favorable weather conditions, increases the effect of protection, and reduce losses of toxic pesticides and soil pollution, as well as surface and ground water. According to research on the effectiveness of pesticide application has been shown to phytotherapeutic effect of pesticides and economy of the process depends to 70% of high-quality applications where 40% depends on the condition and safety of machinery and 30% is professionalism user machines for the application of pesticides. The remaining 30% relates to the use of appropriate pesticides, timeliness of application of protective agents, resistance and others. (Urosevic, 2001).

In Serbia, the use of pesticides is further complicated by inadequate technical level of the application device; testing new machines in operation are generally not enforced, and often

operate machines insufficiently trained persons. (Sedlar et al., 2007; Sedlar et al., 2014). The quality of treatment machines for application in connection with the correctness, adjustability, technical solutions and contemporaneity of machines affect the accuracy of distribution, dosing accuracy and size of the losses (Bugarin et al., 2008).
The introduction of HACCP and "Global GAP" standards in agricultural production guarantees the health safety of products, and the absence of pesticide residues, especially in fresh vegetables and fruits (Koprivica et al., 2015).
Suppression of pathogens and pests is based on the fast and precise distribution and application working fluid, pesticides, respecting working conditions (Višacki et al., 2014). The most important factor spraying machines represent nozzles. They perform important functions such as leakage given amount of liquid per unit time, making disperse liquid droplets of appropriate size and form a stream of appropriate shape (Tadic et al., 2014, Bajkin et al., 2014). Defective nozzles that give higher or lower flow rate due to worn or clogged need to be replaced with new ones.
The EU introduced regulations required inspection of technical means of plant protection directive 2009/128 / EC and 2006/42 / EC, which is the basis of the standard EN 13790 (Banaj et al., 2014). To agricultural producers could export their products to the EU market need to fulfill all the conditions laid down by the "Global GAP" standard (GAP - Good Agricultural Production). This covers the protection of consumers' health, measures to control and limit the use of pesticides and certification of products. Within this standard have significant role nozzles and sprayers that are commonly used as a machine for the application of pesticides in crop farming, orchards and vineyards.
In Serbia in the category "attachable unit" there was a total of 138.084 sprayers and 17.281 atomizers (Radivojevic, 2014). The Republic of Serbia is legally required since 1999 to inspect sprayers and air assisted sprayers. Testing the proper working of machines is carried out in accordance with grading EN 13790 which prescribes methods and equipment used to perform control. Control stations (31 on the territory of Serbia) are equipped with the latest technology to control. The equipment for testing is from a Belgian manufacturer AAMS-Salvarani.
There are two central laboratories in the Faculties of Agriculture in Belgrade and Novi Sad, and other regional laboratories in institutes, agricultural stations and schools. Among the accredited laboratories and the laboratories of the Institute for forage crops in Globoder-Krusevac and is responsible for testing the machines for plant protection in the Rasina district.
The aim of this study was to carry out practical testing of nozzles and the pressure gauges to several types of agricultural sprayer to determine the accuracy and uniformity of flow measurement and flow to nozzles.

Material and method

In July 2015, in a control station in Institute for forage crops in Krusevac-Globoder (Serbia) technical testing of plant protection products was carried out. This is taken to the testing of nozzles and the pressure gauges to six types of agricultural sprayer and determines the correctness and uniformity of flow measurement and flow to nozzles.
Since the equipment was used Nozzle Flow Rate Tester S001 (Figure 1) and controller manometer (Figure 2). Flow measurement is performed at a pressure of 3 bar and rpm PTO 540 min^{-1}. Faulty nozzles represent the biggest problem for the proper functioning of machines for plant protection. In practice, it often happens that the openings in the nozzles clog due to mechanical impurities or water quality or worn during prolonged use, which significantly affects the quality of spraying.
AAMS Salvarani Nozzle Flow Rate Tester is a handheld portable device designed for fast and precise control characteristics and wear nozzles. It is a device that defines the flow of all types

of nozzles mounted sprayers, accuracy class 1%. If it is determined by measuring the flow nozzles deviate by more than ± 10% of Tablet value in a given operating pressure, according to EU standards such nozzles are removed from use.

Figure 1. AAMS-Salvarani Nozzle Flow Rate Tester S001

Control gauge is done by comparing the pressure on the gauge to be tested with the reference pressure gauge manufacturer WIKA. According to European norms gauges that are installed on the machines for plant protection products must have a minimum diameter of 63 mm and the accuracy of the pressure gauge to be tested must be ± 0.2 bar for measuring range up to 2 bar. If it is a larger area measuring deviation may be up to ± 10%.

Slika 2. Tester pressure gauge with "Wika" reference pressure gauge

AAMS Salvarani Pumptester is used to determine the actual capacity of the pump at different pressures. This instrument shows the pressure and flow of liquids.
To check the spraying machine in accordance with EN 13790 is necessary to check about 40 different requests. Some checks are carried out visually, while others need to perform certain tests. Visual inspection is done by reviewing the state hoses, tanks and filters, with the sprayer switched on to work for 1 minute at a pressure of 3 bars. The sprayer is then switched off and check for dripping fluids on nozzles, intestines and anti-drop devices.
Distribution of fluid beneath the wing has a direct impact on the quality of nozzles and distribution of liquid in the operating conditions. The measurement is performed Spray scanner. The spray scanner works automatically under the boom until it performs measurement of the entire length of the boom. To determine the flow of individual nozzles mounted on sprayer used AAMS Salvarani Single Nozzle Tester for atomizer.
Sprayer type A is mounted sprayer manufacturer "Agromehanika" from Kranj, with 19 nozzles. The capacity of the pump sprayer 60 l min^{-1} and the volume of the reservoir for

liquid is 400 l. Sprayer types E is also mounted sprayer "Agromehanika" from Kranj tank volume 330 l, pump capacity 60 l min^{-1}. The sprayer B is driven by volume 400 l Swiss manufacturer "Fischer". Sprayers C, D and F are mounted sprayer 330 l (M 330 Lux) producers "Morava" from Pozarevac. Number of nozzles varied depending on the type of sprayer. Ranged from 16 (C, D, E, F), 19 (A) and 22 (B).

Results and discussion

Faulty nozzles represent the biggest problem for the proper functioning of machines for plant protection. In practice, it often happens that the openings in the nozzles clog due to mechanical impurities or water quality or worn during prolonged use, which significantly affects the quality of spraying.

Test results flow of the working fluid during the test using a sprayer flow meter individual nozzle S001, as shown in Table 1. The table shows the average value of three repetitions. Six different types of sprayer were tested (A, B, C, D, E, and F).

Table 1. The flow of the working fluid crop sprayers

Number of nozzles	The flow of the working fluid crop sprayers (l min^{-1})					
	The type of sprayers					
	A	B	C	D	E	F
1.	1.072	0.824	0.904	1.094	0.545	0.636
2.	0.946	1.242	1.085	1.362	0.632	1.681
3.	1.102	1.154	0.950	1.469	0.691	1.471
4.	1.042	1.257	0.901	1.448	0.690	1.471
5.	1.188	1.255	0.926	1.555	0.769	1.493
6.	1.746	1.232	0.962	1.145	1.118	1.409
7.	1.683	1.314	0.842	1.577	0.777	1.512
8.	1.003	1.061	1.112	1.544	0.784	1.558
9.	1.225	1.048	0.935	1.447	0.768	1.567
10.	1.178	1.306	0.825	1.489	0.796	1.448
11.	1.272	1.508	0.860	1.340	0.968	1.467
12.	1.325	1.353	0.806	1.201	0.634	1.565
13.	1.196	1.461	0.958	1.555	0.744	1.453
14.	1.215	1.258	1.015	1.385	0.791	1.563
15.	1.344	1.244	0.926	1.174	0.625	1.645
16.	1.295	1.184	1.000	0.801	0.619	0.444
17.	1.130	1.144	-	-	-	-
18.	1.439	1.166	-	-	-	-
19.	1.508	1.089	-	-	-	-
20.	-	1.089	-	-	-	-
21.	-	1.001	-	-	-	-
22.	-	1.178	-	-	-	-
Average	1.258	1.198	0.938	1.411	0.747	1.304
CV (%)	16.209	10.611	5.131	11.714	18.885	5.137

The highest variability of nozzles operation was established for the nozzles E and A (CV% = 18.885% and CV% = 16,209) and the lowest for sprayers C and F (CV% = 5.131 and CV% = 5.137%). This indicates that the sprayer E and A not we allowed using all the nozzles on the nozzles while the C and F use of all nozzles was acceptable.

Minimum flow of the working fluid amounted to 0.444 l min^{-1} in F-type nozzles as a result of malfunctioning nozzles, or clogged nozzle. Maximum flow of the working fluid was 1.746 l min^{-1} nozzles in type A which is due to deterioration of the nozzles. The lowest average flow was 0.747 l min^{-1} in E-type sprayer manufacturer "Agromehanika" from Kranj, which was also the latest of the tested sprayer. The highest average flow of the working fluid amounted to 1.411 l min^{-1} in type sprayers D.

Conclusion

Based on the analysis of the current status of implementation of pesticides for agricultural producers in Serbia in Rasinski district can be concluded that the sprayers are very old, except sprayers type D, which is four years old. The nozzle gauges were defective, there were no gauges or used inappropriate pressure gauges, the nozzles A built-in pressure gauge measuring range much larger than necessary.

It is necessary to replace the corresponding faulty gauges pressure gauges to control the proper operation of the nozzles. In some nozzles do not work regulating valves regulating the working pressure sprayer. It was also revealed that the nozzles are not working valves which are used for closing the liquid flow through the boom.

On the sprayer type D membrane air bells at the pump was not correct, causing uneven spray of working liquid. There are significant variations in the flow of the working fluid as a result of wear or nozzles were closed on orifice nozzles. Such nozzles should be replaced with new nozzles respective hole. When working with the correct and well-tuned devices for the application of pesticides manufacturers can realize profit.

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EFFECT OF INDIGENOUS RHIZOSPHERIC ISOLATES PSEUDOMONAS SPP. ON THE INHIBITION OF PSEUDOTHECIA FORMATION AND ASCOSPORES GERMINATION OF VENTURIA INAEQUALIS

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Abstract

It has been showed that apple is host of *Venturia inaequalis*, a very aggressive phytopathogenic fungus known for production scab. In search for ecological ways for prevention and reduction of disease symptoms, biological control using indigenous fluorescent *Pseudomonas* isolates has been offered as a possible solution. The aim of this study was to examine the activity of some indigenous isolates of the genus *Pseudomonas* (B25, Q16) to prevent ascospores germination and pseudothecia forming of pathogenic fungi *V. inaequalis*, in order to minimize the infection potential of pathogens and prevent apple infection. In this experiment we used a series of concentrations of B25 and Q16 isolates of the genus *Pseudomonas* as a biological agent that has shown good antagonistic effect. Copper hydroxide was used as a standard treatment and non-treated plants as a control. Ascospores, which are exempt from the pseudothecia maceration, isolated from samples of owervintering apple leaves, were exposed to the mentioned agents. Readings of results were carried out after 24 and 48 hours.

The obtained results showed that the complete inhibition of ascospores germination achieved at a concentration of 10^8 $CFUml^{-1}$of isolate B25, while a high percentage of inhibition relative to the standard was achieved at a concentration of 10^7 $CFUml^{-1}$of the same isolate.

In prevent of pseudothecia forming best results showed Q16 isolate (24.4% formed pseudothecia), followed by B25 isolate (68.3%) comparing to the cooper hydroxide as a standard with 78.0% formed pseudothecia and control (100%).

Keywords: *antagonisme, apple scab, ascospores*

Introduction

Plant diseases caused by phytopathogenic fungi are one of the major problems in orchard plants in Serbia. Apple scab caused by *Venturia inaequalis* (Cooke) Winter (anamorph *Spilocaea pomi* Fr.) is the most important apple disease. Chemical control with fungicides has been the main measure of disease control (Ivanović, 2001). During the growing season, in order to keep the trees scab free, more than 20 fungicide treatments may be needed (MacHardy, 1996; Ivanović, 2001). In order to protect apple trees from the apple scab, a wide spectrum of different chemical preparations is used from different chemical groups, with different modes of action (preventative, preventative-curative, curative and eradicative) which are often combined in order to enhance their effectiveness or to avoid the development of resistance. Unfortunately, fungicide resistance has been observed in some orchards. Turechek *et al.* (2004) as well as many other researchers warn about the strobilurin fungicides resistance for example.

Therefore, the application of natural microbial antagonists to control apple scab could be a promising alternative to the application of synthetic fungicides (Hossain *et al*. 2009). Biological control represents an efficient alternative or an additional plant protection method which and reduces the need for chemical agents (Postma *et al*., 2003; Welbaum *et al*., 2004).

The agents which are used in biological control are numerous microorganisms, including a special group of rhizospheric bacteria also known as a plant growth promoting rhizobacteria (PGPR) (Cattelan *et al*., 1999; Zehnder *et al*., 2001). Bacteria from the genus *Pseudomonas* represents one of the most widespread and efficient PGPR in biological control of different plant pathogens - fungi, bacteria and viruses (Cattelan *et al*., 1999; Gerhardson, 2002). *Pseudomonas* spp. produce various secondary metabolites with antagonistic effects: antibiotics (phenazine, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin), siderophore, cyanohydrin, tensin, homoserine lactone, cyclic lipopeptide, (Nielsen *et al*., 2002; Raaijmakers *et al*., 2002; de Souza, 2003; Nielsen and Sørensen, 2003). This is the reason why they can be used in the plant protection formulations.

When investigating suitable ecological ways for prevention and reduction of disease symptoms, biological control using indigenous fluorescent *Pseudomonas* isolates has been presented as a possible solution. The key purpose of the biological control is to reduce the inoculum density and the activity of the pathogens that cause disease (Baker and Cook 1974). Therefore, identifying naturally occurring harmless and/or beneficial microorganisms and a subsequent analysis of their biocontrol potential is still a focus of current research (Hyakumachi, 2013).

The aim of this study was to examine the activity of some indigenous isolates of the genus *Pseudomonas* (B25, Q16) in preventing the germination of ascospores and formation of pseudothecia of the pathogenic fungi *V. inaequalis*, in order to minimize the infection potential of this pathogen and prevent apple infection. In this paper, both the germination of ascospores and the formation and maturation (ripening) of pseudothecia were examined.

Material and methods

The *in vitro* conditions were used for the examination of three bacterial isolates of genus *Pseudomonas* (Q16p, Q16L and B25) in order to determine inhibition of *V. inaequalis* ascospores germination. Ascospores were released by maceration of the pathogen's pseudothecia which were extracted from the tissue of overwintered infected apple leaves. Pseudothecia were extracted according to the Aleksić (2006) and Aleksić *et al* (2014) method. These pseudothecia were transferred to the microscopic slide in the drop of suspension of the *Pseudomonas* isolate.

V.inaequalis pseudothecia were further macerated in order to release the ascospores, then covered with a **cover slide** and transferred to the Petri dish with a moist filter paper. These slides were left in darkness to incubate at a temperature of 20°C for 24 to 48 hours, after which the counting of the ascospores' germination was performed. This set up was repeated five times. In order to ascertain germination, 100 ascospores per repetition were examined under the microscope, magnified 400 times. The preparation Funguran OH (copper hydroxide) was used as a standard as well as the untreated control. Three bacterial isolates of the genus *Pseudomonas* (Q16p, Q16L and B25) were used for the examination in a series of five concentrations in the intervals from 10^9 to 10^4 whereas the standard was used in five concentrations of application (0.8; 0.4; 0.2; 0.1 and 0.05%). 0.4% and 0.2% are recommended commercial concentrations. *Pseudomonas* isolates are cultivated on a liquid Waxman medium at 26°C using orbital shaking incubator at 100rpm for 24h, then optimized to 5 x 10^6 $CFUml^{-1}$.

The field experiments were conducted at the start of the vegetative growth in the period from February to April 2014 in an apple orchard PKB-Agroekonomik-Padinska Skela, Belgrade. A Polyvinyl chloride (PVC) net of $1m^2$ was placed tightly over the overwintered leaves, on the orchard floor, under the apple trees of the Golden Delicious cultivar. After that, the three treatments spaced over time, were performed using the following suspensions of bacterial isolates: 2ml x 10^9 $CFUml^{-1}$ of Q16L or 2ml x 10^8 $CFUml^{-1}$ of B25 with a hand sprayer using

100ml of water/m^2 (1000 l/ha). Treatments were carried out on 22 February, 9 March and 25 March. Funguran OH at a concentration of 0.4% (50% copper-hydroxide) and an untreated control were used for comparison.
Following the application of the suspensions, the samples of overwintered leaves were collected on 10 April. From every combination, 50 leaves were sampled after which the pseudothecia were extracted using a binocular lens. Pseudothecia from the sample were transferred to a drop of water on the microscopic slide, macerated and examined under the microscope to determine the stage of pseudothecia formation and maturity according to the Aleksić (2006) and Aleksić *et al*(2014) method. The prepared pseudothecia were examined under the light microscope and categorized in 5 categories (0-K4) according to the scale Aleksić (2006).
The forming and maturation of pseudothecia were calculated using the following formula:

$$P = \frac{K_1 + 2K_2 + 3K_3 + 4K_4}{U * 4} * 100$$

P- percentage of pseudothecia ripening
K- category of pseudothecia ripening
U – total number of examined pseudothecia

The results were analyzed by the variance method and the statistical significance calculated by the Duncan’s test.

Results and discussion

The obtained results showed that the complete inhibition of ascospores germination was achieved at a concentration of 10^8 $CFUml^{-1}$ of the *Pseudomonas* isolate B25, while a high percentage of inhibition relative to the standard was achieved at a concentration of 10^7 $CFUml^{-1}$ of the same isolate (Tab.1; Fig.1).
The isolate Q16p demonstrated the complete inhibition of germination of ascospores in higher concentration of bacteria (10^9 $CFUml^{-1}$), whereas the isolate Q16L has not showed the complete inhibition of germination of ascospores *V. inaequalis* even at the highest concentration of bacterial suspension (tab.1)

Table 1. *V.inaequalis* ascospores germination after 24 and 48 h

	24h							48h						
	I	**II**	**III**	**IV**	**V**	**Ms**	**%**	**I**	**II**	**III**	**IV**	**V**	**Ms**	**%**
K	46	48	61	35	52	**48.4**		84	87	68	94	74	**81.4 i**	
Q16p 10^{9*}	0	0	0	0	0	**0 a**	**100**	0	0	0	0	0	**0 a**	**100**
Q16p 10^{8}	2	1	5	12	18	**7.6**	**84.3**	7	8	20	10	10	**11**	**86.5**
Q16p 10^{7}	0	0	6	10	10	**5.2**	**89.3**	12	8	15	20	10	**13**	**84.0**
Q16p 10^{6}	11	4	8	7	4	**6.8**	**85.9**	3	40	44	40	30	**38.4 hi**	**52.8**
Q16p 10^{5}	6	10	4	5	15	**8 b**	**83.5**	69	36	22	31	34	**38.4 i**	**52.8**
Q16L 10^{9}	8	0	0	0	0	**1.6**	**96.7**	4	2	0	0	0	**1.2 ab**	**98.5**
Q16L 10^{8}	7	0	0	12	2	**4.2**	**91.3**	21	12	4	8	2	**9.4**	**88.4**
Q16L 10^{7}	2	31	7	0	0	**8 b**	**83.5**	2	18	8	0	6	**6.8 abc**	**91.6**
Q16L 10^{6}	0	0	0	19	6	**5 ab**	**89.7**	10	11	19	13	9	**12.4**	**84.8**
Q16L 10^{5}	8	22	19	25	25	**19.8**	**59.1**	24	28	12	24	18	**21.2**	**73.9**
B25 10^{8}	0	0	0	0	0	**0 a**	**100**	0	0	0	0	0	**0 a**	**100**
B25 10^{7}	4	3	2	3	3	**3 ab**	**93.8**	3	5	7	5	6	**5.2 abc**	**93.6**
B25 10^{6}	6	6	4	6	9	**6.2**	**87.2**	12	13	7	10	12	**10.8**	**86.7**
B25 10^{5}	10	9	8	6	9	**8.4 b**	**82.6**	35	31	11	10	18	**21 efg**	**74.2**
B25 10^{4}	18	16	18	20	25	**19.4**	**59.9**	22	19	29	19	23	**22.4**	**72.5**
Fu 8 kg	0	0	0	0	0	**0 a**	**100**	0	2	3	1	2	**1.6 ab**	**98.0**
Fu 4 kg	4	3	5	4	5	**4.2**	**91.3**	7	8	10	9	12	**9.2**	**88.7**
Fu 2 kg	7	7	7	8	7	**7.2**	**85.1**	14	12	10	10	10	**11.2**	**86.2**
Fu 1 kg	12	9	10	11	11	**10.6**	**78.1**	18	23	17	17	19	**18.8**	**76.9**
Fu 0.5 kg	20	20	18	22	17	**19.4**	**59.9**	22	24	24	23	22	**23 gh**	**71.7**

LSD_{005}24h=6.57; LSD_{001}24h=8.71 **LSD_{005}24h=9.14; LSD_{001}24h= 12.12**

* concentration in $CFUml^{-1}$

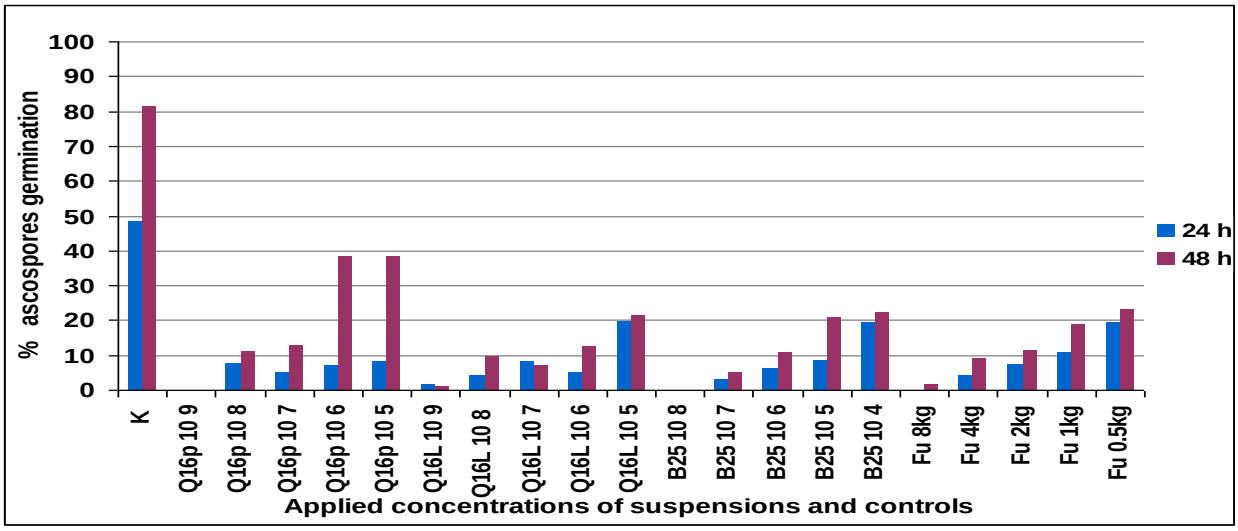

Fig.1 Percentage ascospore germination in different concentrations of *Pseudomonas* suspensions (in $CFUml^{-1}$) and controls after 24 and 48h

Based on achieved results, it can be concluded that the indigenous rhizospheric bacterial isolates of the *Pseudomonas* genus have demonstrated a high level of inhibition of ascospores' germination of phytopathogenic fungi *V.inaequalis*, especially when applied in high concentration of suspensions (10^9 and 10^8 $CFUml^{-1}$) after 24 and 48 hours of activity.
Generally, the isolates B25 and Q16L have shown a sufficient level of inhibition of ascospores' germination in all of the applied concentrations and time exposure, whereas the isolate Q16p which was applied in lower concentrations (10^6 and 10^5) showed the lower level of inhibition of ascospores' germination after 48 hours (Fig.1). Similar results of inhibition of *V. inaequalis* conidia germination were obtained by Hossain *et al*. (2009). These authors determined that the level of inhibition was 73-100% by examining the inhibition of conidia germination with the extracellular protein fractions of antagonistic bacteria *Pseudomonas fluorescens* Bk3.
In the experiment examining the pseudothecia formation, the Q16L isolate showed the best results (24,4% of formed pseudothecia), followed by B25 isolate (68,3%) compared to the cooper hydroxide as a standard with 78,0% formed pseudothecia and a control (100%) (tab.2.).

Table 2. *V.inaequalis*. Pseudothecia forming

	Variants			
Categories	**Control**	**Funguran OH**	**Q16L**	**B25**
non-content		**-**	**3**	**5**
0*	**19**	**14**	**7**	**20**
K1	**-**	**-**	**-**	**1**
K2	**-**	**1**	**-**	**-**
K3	**-**	**5**	**-**	**-**
K4	**3**	**7**	**-**	**2**
discharged	**19**	**5**	**-**	**-**
Total	**41**	**32**	**10**	**28**
maturity (P)	**53.7**	**35.1**	**0**	**8.0**
Formed (%)	**100**	**78.0**	**24.4**	**68.3**

*0 – Pseudothecia without ascospores; K1-up to 25% of asci with ascospores formed; K2-26-50% ; K3-51-75%; K4=76-100%

These results are the first reported and very encouraging results of the inhibitory effect of rhizospheric bacteria on the *Venturia inaequalis* pathogen. Scientific research on the use of biological control for the purpose of preventing apple scab infection or as a contributor to the integral plant protection control is limited. Only a few authors have found some antagonistic effect on *Venturia* of epiphytic *Pseudomonas* bacteria (Singh et al, 2004). Köhl *et al.* (2009) showed that an application of conidial suspensions of *Cladosporium cladosporioides* caused a reduction in the production of *Venturia inaequalis* conidia.

Further research could include the effect of *Pseudomonas* suspensions on the ripening of pseudothecia as well as their formation and whether this can reduce the level of infection even more. On a larger scale, measuring the effect of the bacterial suspension in field conditions over a longer period of time would show if the use of biocontrol is a truly feasible alternative to chemical control of *Venturia inaequalis*.

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ALUMINA POWDERS AS NOVEL NON-TOXIC INSECTICIDE AGAINST BEAN WEEVIL (Acanthoscelides obtectus Say)

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Abstract

Alumina powders were obtained by auto combustion method and calcined for 1 h at temperatures ranging from 1000ºC to 1200ºC. X-ray powder diffraction analysis revealed an overwhelming presence of γ–Al_2O_3 for the powder calcined at 1000ºC, while at 1200ºC only the presence of α–Al_2O_3 was detected. Insecticide properties of these powders were investigated on bean weevil (*Acanthoscelides obtectus* Say). Mortality of treated and untreated male and female adults was checked in a period of 7 days. The powder calcined at 1200ºC was the most efficient, and 100% mortality of both male and female insects were reached after 7 days of exposure. It was also observed that insecticide efficiency increased with the increase of α–Al_2O_3 phase present in the powders and the female insects were less susceptible to the powders' toxicity in general. Scanning electron microscope analysis of the treated adults with Al_2O_3 indicated that mortality of bean weevils can be related to adsorption of the powders onto their exoskeleton. Development of F_1 progeny was similar for all the powders and the number of hatched larvae was significantly reduced in comparison with the untreated seeds, while the percentage of damaged seeds dropped from 70% to 15%. These results suggest that relatively cheap and non-toxic α–Al_2O_3 powders can be considered for use in pest control, especially in organic seed production.

Keywords: alumina; pest control; bean weevil, *Acanthoscelides obtectus*.

Introduction

The bean weevil (*Acanthoscelides obtectus* Say) is considered as one of the most serious stored bean pests worldwide (Jovanović et al., 2007). Its oviposition and growth are continuous, and the larvae feed on the seeds. After emergence from the seeds, the adults reproduce either in the field or in the stored seeds in a continuous cycle (Ayvaz et al., 2010). Since bean is one of the most important nutritive sources in developing countries, its protection in stored conditions has been attracting much attention in past decades. Protection of agricultural stored products against insect pests is of utmost importance to secure a continuous and safe food supply all over the world (Pemonfe et al., 1997). Conventional treatments have been used for this purpose, but for the last decades there have been numerous reports of environmental contamination by use of toxic pesticides, mostly organophosphorous and pyrethroid compounds (Nikpay, 2006). Their use has been constantly challenged by resistance of insects, consumer's demand for pesticide-free products and restrictive governmental regulations (Subramanyam et al., 1994). This led to replacement of the existing chemical insecticides by a new class of non-toxic, nanostructured inorganic compounds, such as silica, alumina, diatomaceous earth and inert dusts (Barik et al., 2012; Reynolds et al., 2009; Stadler et al., 2010; Stadler et al., 2012). Especially these compounds have been reported to be effective in control of stored product insects (Nikpay et al., 2006; Stadler et al., 2010; Stadler et al., 2012). This approach was successfully demonstrated in pest control of

rice weevil (*Sitophilus oryzae* L.) and lesser grain borer (*Rhyzopertha dominica* F.) by nanostructured alumina (Stadler et al., 2010; Stadler et al., 2012).
The cause of mortality of insects by applying these inert inorganic powders has not yet been fully clarified. However, this approach being cost effective and environmentally friendly is worth further investigations and optimization of the existing formulations. Thus, in this study, Al_2O_3 powders obtained after calcination at different temperatures were tested as potential insecticides against *A. obtectus*. The comparison of insecticidal properties was observed for male and female adults and F_1 progeny.

Materials and methods

Synthesis and characterization of alumina powders: 0.03 moles of aluminium-nitrate nonahydrate ($Al(NO_3)_3 \cdot 9H_2O$, "Centrochem", Serbia) was dissolved with citric acid monhydrate ($C_6H_8O_7 \cdot H_2O$, „Lachner", Czech Republic) in minimal amount of distilled water, whereas the citrate to nitrate molar ratio was set at 0.8. pH value was kept at 8 by adding ammonium hydroxide (NH_4OH, „Lachner", Czech Republic) into the solution. First, the solution was heated at 80 ºC with constant stirring until a viscous gel was formed. Then, the temperature was raised up to 200ºC to start the autocombustion reaction. The resulting black precursor powder was collected and calcined at 1000ºC, 1100ºC and 1200ºC for 1 h in a chamber oven. Phase content of the powders was determined by X-ray diffraction (XRD, RIGAKU® RINT 2000) analysis using a CuK_α radiation ($\lambda_{CuK\alpha} = 1.54178 \times 10^{-10}$ m) from 20º to 80º 2θ angle.
Test insects: Male and female insects of *A. obtectus* were reared separately under controlled conditions (27ºC±1ºC, relative humidity 65%±5% and photoperiod L16:D8) and were 48 h old prior to testing. The insects were provided from the Institute for Biological Research "Siniša Stanković" (Belgrade, Serbia).
Lethal effect of the alumina powders: Al_2O_3 powders were mixed with 40 g bean grains by rotary shaker (Multifix GmbH, Germany) for 10 min, to form 1% mass concentration. The mixtures were further divided into four smaller portions of 10 treated grains with 10 male, or 10 female insects placed separately in a 90/14 mm Petri dish. Additional four control portions contained only untreated grains with insects. All tests were carried out simultaneously under controlled conditions and mortality rate was tracked after each day, during 7 days.
Effectiveness of powders on reduction of number of individuals in F1 progeny: Al_2O_3 powders were mixed with 40 g bean seeds in 200 ml glass jars by rotary shaker (Multifix GmbH, Germany) for 10 min, to form 1% mass concentration. Each batch was divided into four smaller samples with 10 g treated seeds, which were placed in 90/14 mm Petri dishes together with 10 female and 10 male insects. Control sample was prepared in the same way, but contained only untreated seeds. Each testing was repeated four times. After 15 days dead insects were removed from the Petri dishes and the development of F_1 progeny was investigated. The number of F1 progeny produced was recorded after 30 days. The experiments were carried out under controlled laboratory conditions (T=27ºC ± 0.5ºC, RH= 65%, L16:D8).
SEM analysis: In order to determine any changes of the exoskeleton, the tested insects were analyzed by scanning electron microscopy (SEM, TESCAN Vega TS5130MM).

Results and discussion

XRD analysis of the powders calcined at different temperatures was presented in Figure 1. The changes in diffraction patterns indicated a gradual transformation from γ–Al_2O_3 to α–Al_2O_3 as the calcination temperature increased from 1000ºC to 1200ºC.

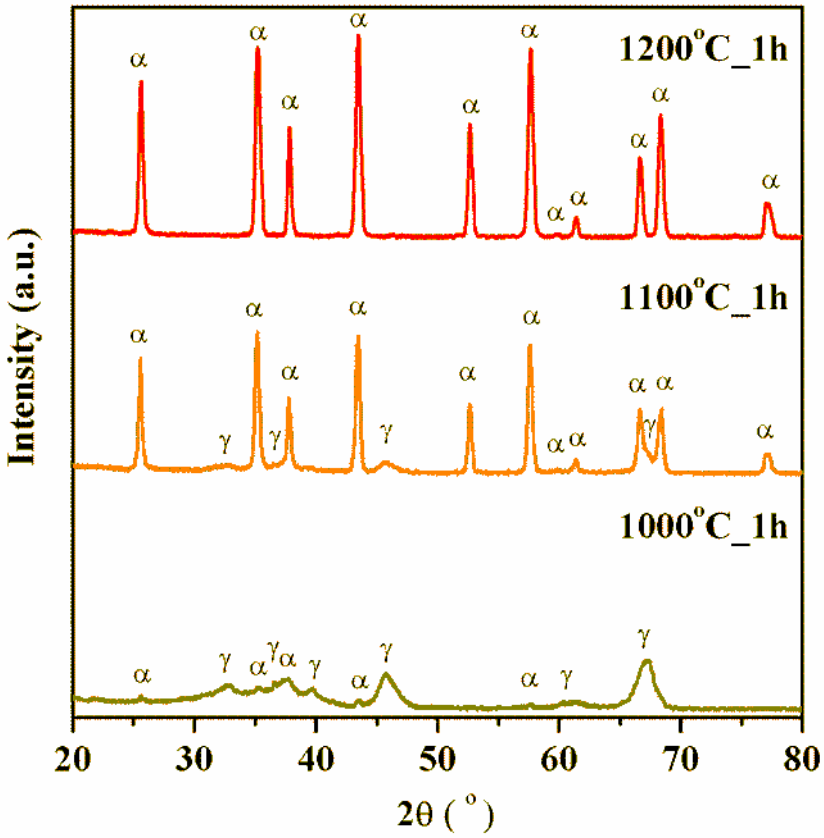

Figure 1. XRD patterns of alumina powders calcined at 1000–1200ºC for 1h.

The results of the lethal effect of the alumina powders on treated and untreated male and female adults in a period of 7 days were presented in Figure 2. After 24 hours all insects were alive, only after 48 h the first mortality cases were observed. Al_2O_3 powder calcined at 1200ºC began to be lethal against male adults after 48 h. After 72 h, mortality was 40%, after 96 h, more than 60%, after 120 h, 70% and after 144 h, mortality reached 100%. In the case of females Al_2O_3 powder calcined at 1200ºC began to be lethal after 72 h, with mortality of 20%. The maximal lethal effect of almost 100% was reached after 168 h. Al_2O_3 powders calcined at 1100ºC and 1000ºC showed their lethal effect on male adults after 72 h and 96 h. They showed maximal lethal effect after 168 h, 100% and 97.5%, respectively. These powders exhibited lethal effect on female adults first after 120 h and maximal lethal effect they showed was after 168 h, 92,5% and 62,5%.

It was clear that pure α–Al_2O_3 powder obtained after calcination at 1200ºC was the most lethal against both male and female adult insects. Taking into account untreated adult insects, it is clear that female insects live a bit longer, probably due to their final role in laying eggs that were previously fertilized. This trend is also observed for treated insects, leading to a conclusion that females are less overall susceptible to alumina powders. Furthermore, lethal effect decreased almost monotonously with the increase of γ–Al_2O_3 content in the powders for female insects, while the powders calcined at 1000ºC and 1100ºC showed no significant difference in their insecticidal activity against male insects.

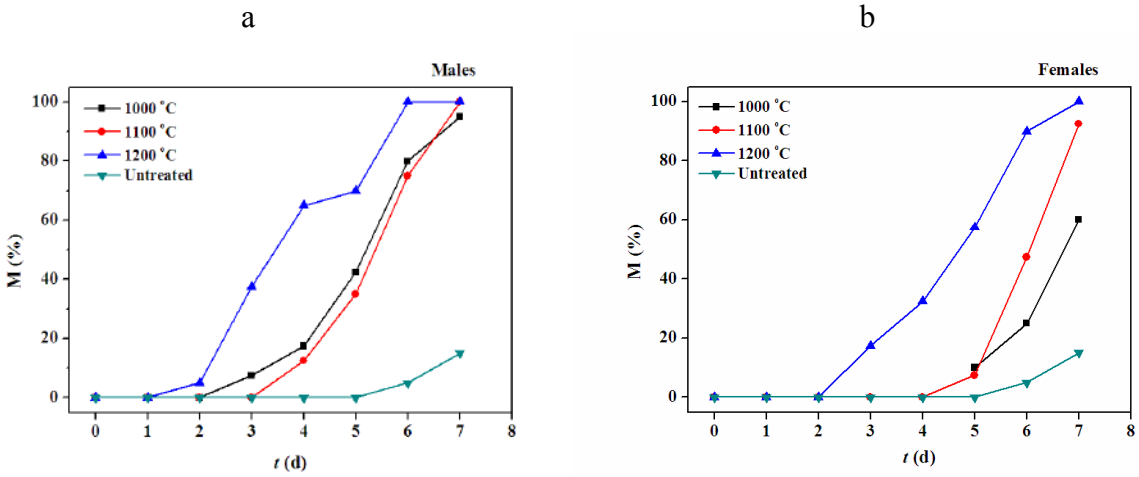

Figure 2. Lethal effect of powders against male (a) and female insects (b) in a period of 7 days.

Compounds like α–Al_2O_3 powder, if attached to an exoskeleton of insects, are able to adsorb cuticular lipids, which can cause dehydration of insects and their death, consequently (Stadler et al., 2012). SEM analysis of untreated and insect treated with Al_2O_3 was presented in Figure 3 and it is easy to notice how Al_2O_3 particles adhere to the exoskeleton of the treated imago. Thus, mortality rate is certainly related to a degree of adhesion of these particles onto the insect exoskeleton, keeping in mind that α–Al_2O_3 are inert and non-toxic. Degree of powder adhesion depends on factors such asspecific surface area and concentration of powders, humidity, temperature and insect pubescence (Fields et al., 2000; Stadler et al., 2012). On the other hand, by optimizing these parameters it is possible to raise efficiency of alumina powders, and accordingly, even lower concentrations could provide satisfying efficiency. Thus, these results suggest that α–Al_2O_3 powders, being the most effictive against *A. obtectus*, can be considered as an alternative in pest control of the stored products and in organic seed production, as well (Krnjajić, 2003; Obradović and Krnjajić, 2003).

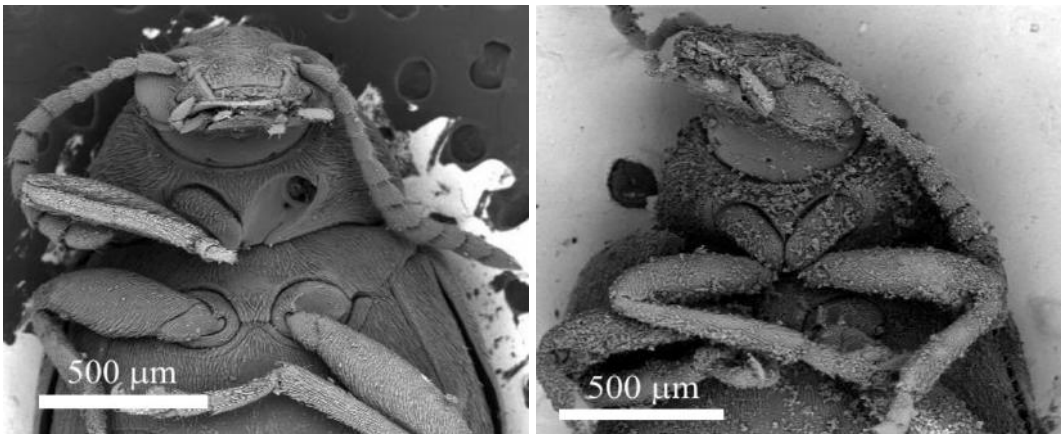

Figure 3. SEM images of untreated (left) and treated insect with Al_2O_3 (right).

When it comes to the lethal effect of alumina powders on F_1 progeny, again the powder calcined at 1200ºC exhibited the highest efficiency (Figure 4), but there is a slight difference in their efficiency. The number of larvae hatched from eggs under treated conditions was

reduced about 75%. The percentage of damaged seeds as a consequence of larval activity is also significantly reduced (dropped from 70% to 15%), which confirmed the pesticide effect of alumina powders against *A. obtectus*. These results clearly serve as both qualitative and quantitative indicators of pest control efficiency of the stored products. This efficiency can be ehanced by increasing powder concentration, since 1% is probably too low for more robust *A. obtectus* compared with *Sitophilus oryzae* (L.), or *Rhyzopertha dominica* (F.), whereas this concentration was used as maximal (Stadler et al., 2010; Stadler et al., 2012).

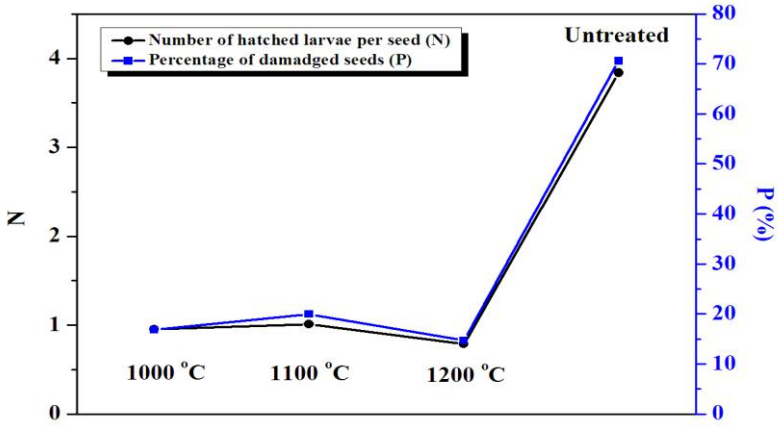

Figure 4. Development of F_1 progeny (a) and percentage of damaged seeds (b).

Conclusions

In this study comparative lethal effect of various Al_2O_3 powders calcined at different temperatures, 1000ºC, 1100ºC and 1200ºC for *Acanthoscelides obtectus* was successfully carried out. Al_2O_3 powder calcined at 1200ºC was the most efficient against adult insects, reaching 100% mortality after 6 days of exposure for males and 7 days for females, compared with 17,5% (males) and 12,5% (females) mortality of the untreated insects after 7 days. On the other hand, all the Al_2O_3 powders exhibit similar lethal effect level against larvae insects in F1 generation and percentage of the damaged seeds was significantly reduced. Al_2O_3 is also promising due to its cost-effectiveness and solid effeciency against *A. obtectus*. Their efficiency can be enhanced by increasing their concentration and/or by optimizing synthesis parameters with aim to obtain powders with appropriate particle size and increased specific surface area.

Acknowledgements

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CHARACTERISTICS OF FATTY ACIDS AND ESSENTIAL OIL FROM SWEET AND BITTER FENNEL FRUITS GROWING IN SERBIA

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Abstract

Fennel (*Foeniculum vulgare* Mill.) is a medicinal plant as well asa flavoring agent in food, cosmetics and pharmaceutical products. There are two commercial types: sweet fennel (var. *dulce*) and bitter fennel (var. *vulgare*). These two fennel types were cultivated in Serbia during 2014, on an experimental field in Mošorin (Vojvodina province, Serbia). Fatty oil content was determined by Soxhlet extraction, while fatty acid composition was determined by esterification and GC analysis. Essential oil content was determined by hydrodistillation, whereas the composition of obtained essential oils was investigated using Gas Chromatography and Mass Spectrometry analysis.

Oil content in seeds of sweet and bitter fennel was3.97% and 6.39%, respectively. The major compound in both fennel oils was saturated myristic fatty acid (64.85% and 47.86%, respectively), followed by unsaturated C18:1 (21.71% and 25.03%, respectively). In sweet fennel these two fatty acids participated with 86.56%, and other five fatty acids (C4:0, C10:0, C12:0, C16:0 and C18:22) were present from 1.45 to 4.22%. In bitter fennel C14:0 and C18:1 fatty acid participated with 72.89%. Another significant fatty acid was capric acid (17.83%), followed by polyunsaturated C18:2 (4.41%),while other four fatty acids (C4:0, C6:0, C12:0 and C16:0) were present from 0.35 to 1.97%.Essential oil content in sweet and bitter fennel was 7.1% and 8.0%, respectively. The major compound in both fennel essential oils was *trans*-anethole (80.0% and 67.1%, respectively). In sweet fennel the second major compound was limonene (11.4%), followed by fenchone (3.9%) and methyl chavicol (2.7%), while in bitter fennel that was fenchone (22.6%) followed by *α*-pinene (3.3%).

Key words: *Foeniculum vulgare*, myristic acid, *trans*-anethole

Introduction

Fennel (*Foeniculum vulgare* Mill.) is a medicinal and aromatic plant from Apiaceae family. Essential oil of fennel fruits is used as a flavoring agent in food products such as beverages, bread, pickles, pastries and cheese because of its aroma (Shahat et al., 2011). Apart from essential oil, plants from this family are rich in fatty oils, which also have great biological potential (Öztürk et al., 2014).

Fennel fruits are used as raw material in cosmetic and pharmaceutical industry (Kooti et al., 2015). It is used in traditional medicine worldwide, but it is also a very popular plant in modern phytotherapy (Rahimiand and Ardekani, 2013), because of its antioxidative (Oktay et al., 2003; Chang et al., 2013), hepatoprotective (Özbek et al., 2003, Rabeh and Aboraya, 2014) and anticancer activity (Mohamad et al., 2011). It is also known that fennel can reduce cholesterol levels (Rezq, 2013), and the seed extracts can be successfully used in the treatment of obesity (Garg et al., 2011). Fennel also posses hypoglycemic effect (Dongare et al., 2010), and it can be used as a natural antidiabetic drug in the pharmaceutical industry (El-Soud et al., 2011).

The objective of the present work was to determine the fatty acid and essential oil composition of two fennel varieties grown in Serbia.

Material and method

Plant material. Sweet fennel (*Foeniculum vulgare* var. *dulce*) variety “Fino”(Austrosaat AG, Austria) and bitter fennel (*Foeniculum vulgare* var. *vulgare*) variety “Vojvodjanski” (Institute for medicinal plant research “Dr Josif Pančić”, Serbia) were grown during 2014 at an experimental field in Mošorin (45°18' N, 20°09' E, altitude 111m), Vojvodina province (Serbia).

Fatty acids analysis. Fatty oil content was determined by Soxhlet extraction. Fatty acid methyl esters were prepared from the extracted lipids using the method based on 14% boron trifluoride/methanol solution, as the recommended method for this type of substrates (Verešbaranji, 1996). Nitrogen gas was used for drying and removing solvents from fatty acid methyl esters. Obtained samples were analyzed by a GC Agilent 7890A system with FID, autoinjection module for liquid, equipped with fused silica capillary column (SP-2560, 100m x 0.25mm, I.D., 0.20µm). Helium was used as the carrier gas (purity >99.9997 vol%, flow rate =1.26ml/min). The fatty acids peaks were identified by comparison of retention times with retention times of standards from Supelco 37 component fatty acid methyl esters mix and with data from internal data library, based on previous experiments. Results were expressed as mass of fatty acid or fatty acid group (g) in 100g of fatty acids.

Essential oil analysis. Essential oil was obtained by hydrodistillation using the Clevenger-type apparatus from crushed mature fruits. The oil quality was assessed through analysis by combined Gas Chromatography and Mass Spectrometry. GC-MS analysis was performed by using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD), in positive ion electron impact (EI) mode. The separation was achieved by using Agilent 19091S-433 HP-5MS fused silica capillary column, 30m x 0.25mm i.d., 0.25µm film thickness. The GC oven temperature was programmed from 60°C to 285°C at a rate of 4.3°C/min. Helium was used as the carrier gas; inlet pressure was 25 kPa; linear velocity was 1ml/min at 210°C. Injector temperature: 250°C; injection mode: splitless. MS scan conditions: source temperature, 200°C; interface temperature, 250°C; energy, 70 eV; mass scan range, 40-350 amu. Identification of components was done on the basis of retention index (RI), their retention times (RT) and by comparison with reference spectra (Wiley and NIST databases).

Results and disscusion

Oil content in seeds of sweet and bitter fennels was 3.97% and 6.39%, respectively (Table 1). The major compound in both fennel oils was saturated myristic fatty acid (64.85% and 47.86%, respectively), followed by unsaturated C18:1 (21.71% and 25.03%, respectively). In sweet fennel these two fatty acids participated with 86.56%, and other five fatty acids (C4:0, C10:0, C12:0, C16:0 and C18:22) were present from 1.45 to 4.22%. In bitter fennel C14:0 and C18:1 fatty acid participated with 72.89%. Another significant fatty acid was capric acid (17.83%), followed by polyunsaturated C18:2 (4.41%), while other four fatty acids (C4:0, C6:0, C12:0 and C16:0) were present from 0.35 to 1.97%.

In Turkey, the obtained oil content in sweet and bitter fennel was 12.22% and 14.41%, respectively (Coşge et al., 2008). The C18:1, C18:2, and C16:0 were the principal fatty acids which constituted around 97% of the total oil content. The ratios of essential oil from sweet and bitter fennel were found similar (average 3.00%).

Table 1 Fennel fatty acids

Fatty acid	R.t. (min)	sweet fennel	bitter fennel
C4:0	10.102	1.55	1.24
C6:0	10.546	nd	0.35
C10:0	13.874	3.85	17.83
C12:0	17.041	2.37	1.97
C14:0	21.073	64.85	47.86
C16:0	25.526	1.45	1.31
C18:1	30.985	21.71	25.03
C18:2	32.692	4.22	4.41
Fatty oil content		**3.97**	**6.39**

R.t. – Retention time; nd – not detected

Essential oil content in sweet and bitter fennels was 7.1% and 8.0%, respectively (Table 2). According to our Pharmacopeia, essential oil content in fruits of sweet fennel need to be more than 2%, while in bitter fennel fruits more than 4% (Ph. Jug V, 2001).
The major compound in both fennel essential oils was *trans*-anethole (80.0% and 67.1%, respectively). In sweet fennel the second major compound was limonene (11.4%), followed by fenchone (3.9%) and methyl chavicol (2.7%), while in bitter fennel that was fenchone (22.6%) followed by *α*-pinene (3.3%).

Table 2 Fennel essential oil

Compounds	R.t. (min)	RI	sweet fennel	bitter fennel
α-pinene	5.816	936	1.0	3.3
camphene	6.225	951	tr	0.2
sabinene	6.911	976	tr	0.1
β-pinene	7.024	980	tr	0.2
myrcene	7.398	994	0.3	1.0
α-phellandrene	7.867	1008	0.1	0.3
p-cymene	8.553	1028	tr	tr
limonene	8.688	1031	11.4	2.2
β-ocimene	9.013	1040	tr	tr
γ-terpinene	9.784	1060	0.2	0.6
cis-sabinene hydrate	10.098	1062	nd	tr
fenchone	10.942	1084	3.9	22.6
camphor	13.227	1142	tr	0.4
methyl chavicol	15.567	1200	2.7	2.0
carvone	17.436	1241	0.4	nd
cis-anethole	18.033	1248	tr	tr
trans-anethole	19.531	1282	80.0	67.1
Essential oil content			**7.1**	**8.0**

R.t. – Retention time; RI – Retention indices on HP-5 MS capillary column; tr – compound present in traces (less than 0.1%); nd – not detected

The compound with the highest value in the sweet and bitter fennel from Turkey was *trans*-anethole as 95.25% and 75.13%, respectively (Coşge et al., 2008). While methyl chavicol was found in bitter fennel oil in a remarkable amount (15.51%), sweet fennel oil contained small amounts of methyl chavicol (2.87%). Fenchone was found in less than 1% in sweet and around 5% in bitter fennel.
There are a lot of factors which can influence the essential oil content and composition. Growing technology such as date of sowing, plant density, apply fertilizers etc. have a

significant influence on essential oil content (Mohamed and Abdu, 2004; Ayub et al., 2008; Khorshidi et al., 2010). Alternatively, very important factors are the environmental and ecological characteristics of the particular growing region (Shahat et al., 2012). However, the content of *trans*-anethole in essential oil from fennel grown in Romania varied between 60.4-77.6%, while fenchone was present from 8.6-13.1%, and methyl chavicol 2.5-8.3% depending on the year of cultivation (Aprotosoaie et al., 2010).

Conclusion

Fatty oil and essential oil content in bitter fennel seed is higher than in the sweet variety. The major compound in both fatty oils was saturated myristic acid, followed by unsaturated C18:1, while the major compound in both essential oils was *trans*-anethole, followed by limonene in sweet fennel, and fenchone in bitter fennel.

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Original scientific paper
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ANTIFUNGAL ACTIVITIES OF DIFFERENT ESSENTIAL OILS TO MARIGOLD SEEDS MYCOPOPULATIONS

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Abstract

This study investigates the possibility of biological control of fungal species isolated from marigold seeds using essential oils from medicinal plants: mint (*Mentha spicata* L.), sage (*Salvia fruticosa* L.), rosemary (*Rosmarinu sofficinalis* L.), anise (*Pimpinella anisum* L.), bitter fennel (*Foeniculum vulgare spp. piperituum* L.) and myrtle (*Myrtus communis* L.).

Two fungal species isolated from marigold seeds: *Botritiscinerea* and *Sordaria fumicola*, were used in this experiment. The minimum inhibitory concentrations (MIC) were determined by micro-dilution method using selected essential oils (EOs). MIC was determined by a modified micro-dilution method in 96 well microtiter plates. Spores were added to each well with 90 μL potato dextrose medium with appropriate dilutions of the EO 10 μL fungal inoculum ($1.0x10^5$-$5.0x10^5$). All experiments were performed in duplicates and repeated four times. The microplates were incubated for 72h at 28°C. The MIC was defined as the lowest concentration of essential oils (EO) which completely inhibited the visible fungal growth. Fluconazole was used as positive control.

Essential oils MIC values were in the range of 0.00325-7.75.0 mg/mL. Among thetestedoils *Salvia fructicosa* EO proved to be the best inhibitor of *B.cinerea* (MIC 0.003mg/mL) and *Myrtus communis* EO to the *Sordaria fumicola* (MIC=0.032mg/mL). The tested fungi were observed to have a susceptibility to all selected essential oils. These results suggest the possibility for application of the EOs in biological control of marigold production.

Key words: *Antifungal activity, Essential oils, MIC, marigold*

Introduction

The plant disease control in agriculture using the antimicrobial chemicals is very often associated with serious problems: toxicity, carcinogenic and teratogenic effect on people and fungal resistance to the fungicide, justifying the search for new strategies in plant protection (Rapp, 2004). The essential oils and other products of especially medicinal plants become a potential antifungal agents in recent years (Bouchra et al. 2003; Carmo et al. 2008; Tavassoli et al. 2011). The phytopatological fungi are very often a casual agents of numerous diseases during the cultivation of medicinal plants. The presence of the fungi in medicinal plants reduces their quality (Essono et al. 2007). On the other hand the medicinal plants are a very rich source of a biologically active compounds including antifungal activity (Kumar et al. 2007; Carmo et al. 2008; Arrebola et al.2010; Bouzennaans Krichen, 2013; Stević et al. 2014). The antimicrobial activity of selected species has already been demonstrated (Maksimovic et al. 2005; Carmo et al. 2008; Tawassoli et al. 2011; Cosic et al. 2010; Istianto and Emilda, 2011; Vitoratos et al. 2013; Mahilrajan et al. 2014).Therefore, the application of essential oils is an alternative, biological control measure.

This study was undertaken to investigate the inhibitory effects of a number of essential oils (EOs), against two phytopathogenic fungi isolated from marigold seeds.

Material and methods

Essential oils

EOs of selected plants (listed in Table 1.), were obtained by hydro-distillation in a Clevenger-type apparatus. The obtained essential oils were stored in sealed glass bottles, protected from the light by wrapping in aluminium foil and storing at -18^{o}C.

Table 1. Essential oils

No	Essential oil	Plant origin	Origin	Used Parts
1	Mint	*Mentha spicata* L.	Mersin (Turkey)	leaves
2	Sage	*Salvia officinalis* L.	Mersin (Turkey)	Leaves+flowers
3	Sage	*Salvia fruticosa* L.	Sarajevo (BIH)	Leaves
4	Rosemary	*Rosmarinus officinalis* L.	Mersin (Turkey)	Leaves
5	Anise	*Pimpinella anisum* L.	Mersin (Turkey)	Fruit
6	Bitter (fennel)	*Foeniculum vulgare* spp. *piperituum* L.	Mersin (Turkey)	Fruit
7	Myrtle	*Myrtus communis* L.	Mersin (Turkey)	Leaves

Fungi

Antifungal activity was tested using two fungal species isolated from marigold seeds during 2014 identified as: *Botritis cinerea* and *Sordaria fumicola*.

Antifungal assay in vitro

To investigate the antifungal activity of essential oil, modified micro-dilution method was used. A minimum inhibitory concentration (MIC) was determined using 96 well microtiter plates (Douk et al. 1995; Nikolić, 2014). The fungal spores were washed from the surface of agar plates with sterile 0.75% saline containing 0.1% Tween 80 (vol/vol), filtered and adjusted to a concentration of approximately $1.0x10^5$ - $5.0x10^5$ spores per ml using a hemocytometer. Fungal inoculum was added to each well with 90 µL potato dextrose medium with appropriate dilutions of the EO 10 µL. All experiments were performed in duplicates and repeated four times. The microplates were incubated for 72 h at 28 °C. The MIC was defined as the lowest concentration of essential oils (EO) which completely inhibited the visible fungal growth. Fluconazole was used as positive control.

Statistical analysis

The results of the minimal inhibitory concentrations (MIC) were processedby Duncan’s multiple range tests. Analysis of the variance was performed on MIC data of seven oils on 2 pathogenic fungi. Significance was evaluated at $p<0.05$ for all tests. Statistical analyses were done by procedures of STATISTICA v.7 (StatSoft, Inc.) and IBMSPSS Statistics v.20 (SPSS, Inc.).

Results and discussion

The obtained results according to the micro-dilution method referring the antifungal activity of the essential oils are shown in Table 2. and Fig. 1.

The essential oils demonstrated varying levels of antifungal effects against fungal pathogens. Essential oils MIC values were in the range of 0.003-10 mg/mL. Among the oils tested, myrtle EO proved to be the best inhibitor of the both tested fungal isolates in concentrations between 0.0325 and 0.775 mg/mL.

Tab. 2 Antifungal activity of the essential oils expressed through the minimal inhibitory concentration

Essential oil	*Botritis cinerea*	*Sordaria fujicola*
Mentha spicata L.	1d*	0,55d
Salvia officinalis L.	10a	0,55d
Salvia fruticosa L.	0,00325d	5,5bc
Rosmarinus officinalis L.	0,325d	7,75ab
Pimpinella anisum L.	0,2555d	5,5bc
Foeniculum vulgare spp. *piperituum* L.	1d	3,25cd
Myrtus communis L.	0,775d	0,0325d
Fluconazole	3,825cd	4,175bcd

*Values of MIC, followed by the same letter are most significantly different ($p<0,05$), according to Duncan's multiple range test.

Salvia fructiocosa EO showed the strongest inhibitory effect on the species *Sordaria fujicola* (MIC: 0,55mg/mL, and low to the *Botrytis cinerea* (MIC: 10 mg/mL). *Botritis cinerea* was more sensitive (average MIC: 1.91 mg/mL) than *Sordaria fujicola* (average MIC: 3.30mg/mL) to the tested EOs.

The antifungal activity of selected oils to the *B.cinerea* referred to in this paper, are in agreement with results of the other researchers. According to the data from Combrinck et al. (2011) an essential oil from *Mentha spicata* has demonstrated antifungal activity to the numerous fungal species, especially to the *Botrytis cinerea.*

EOs used in this study have already shown high inhibitory effect against more fungal species, e.g. anise oil was found to inhibit the growth of *Fusarium* species in concentrations between 0.7 and 2.2 mg/mL (Stevic et al. 2014), bitter fennel EO has high inhibitory effect against *F. oxysporum* (Özcan et al. 2006). In our study, the MIC values of anis EO was 0,2555 to 5,5 mg/mL to the *B.cinerea* and *S.fumicola* respectively.

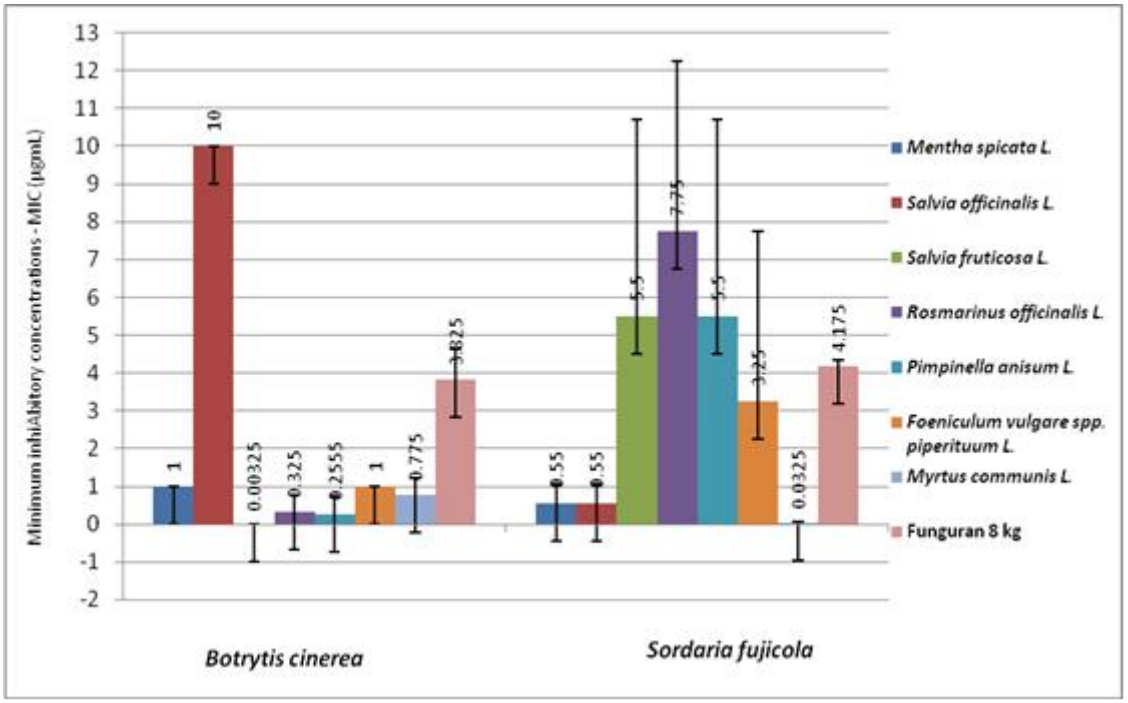

Fig. 1. Antifungal activity of the essential oils is expressed through the minimal inhibitory concentrations (mg/mL) to the *Botrytis cinerea* and *Sordaria fujicola*

This present study provides scientific evidence that different medicinal species EOs can be used as potential sources of bioactive substances. The obtained results could be useful from the practical point of view. An opportunity to test these results *in vivo*, by seed dressing with EOs investigated here, can lead to better monitoring of seedlings' health.

Conclusions

The essential oils of mint, sage, rosemary, anise, bitter fennel and myrtle exhibited good antifungal activity against both tested organisms, known as marigold pathogens. The obtained results in this work suggested that specified essential oils can be applied to prevent growth of phytopathogenic fungi. Further studies on the combined effects of many local plant essential oils could lead to better protection the marigold seeds.

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DISSIPATION OF HERBICIDE NICOSULFURON IN SOIL UNDER FIELD CONDITIONS

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Abstract

Contaminated soil represents a serious environmental problem as the pesticides can be transported to other environmental systems such as surface and ground water, as well as they can cause phytotoxicity on the following crops. Length of pesticide persistence in the environment depends on numerous factors. This paper presents results of nicosulfuron dissipation rate studies in soil of the chernozem type, carried out under field conditions. Field trial was conducted according to OEPP standard methods for experimental design and data analysis. Nicosulfuron OD 40 g/l has been applied post-emergence on a corn crop at the BBCH 12-18 corn growth stage, at the manufacturer's recommended concentration of 44 g/l. Soil samples were collected before and immediately after pesticide application, and every two weeks till harvest, from the surface soil layer of 0-30 cm. Nicosulfuron in soil samples was determined by HPLC-DAD. For the extraction of nicosulfuron, QuEChERS original method EN 15662 was modified. The validation study was performed according to SANCO/825/00 rev.8.1 16/11/2010. The average value of the recoveries at 0.05 mg/kg and 1.0 mg/kg was 83.9%, with the relative standard deviation of 1.5%. The LOD of the analytical method was 0.01 mg/kg and the LOQ was 0.05 mg/kg. In the first two weeks after application, nicosulfuron dissipation was the most rapid (37.9%). Calculation of the results show that nicosulfuron half-life ($t_{1/2}$) in the field soil under corn was 6.93 days.

Key words*: HPLC, nicosulfuron, dissipation,* $t_{1/2}$

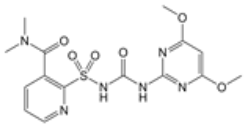
Introduction

Residues of some herbicides in the soil can be beneficial during the season of application for control of later flushes of target weeds, thereby reducing potential competition with the crop being grown (Geisel, 2007). However, soil residual herbicides have been found to persist in the soil into the following year, potentially reducing yields of sensitive crops grown in rotation (Cobucci et al., 1998; Johnson et al., 1993). The amount of herbicide that sorbs to the soil and the rate it can desorb back into the soil solution determines the overall phytotoxicity of herbicide (Geisel, 2007).

Stability of herbicides in soil depends on many factors, physical and chemical properties of herbicides, climatic conditions and soil properties during the vegetation period. Microorganisms activity, constant temperature and soil moisture changes have a great influence on herbicide fate in the soil (Vicari et al., 1994). Very important parameter that defines herbicide potential of persistence in the soil is ($t_{1/2}$), i.e. half-life period. One of the factors by which the potential herbicide mobility in the soil is valuated is relative mobility index of herbicides (Rf) (Helling and Turner, 1968). At very low dose, sulfonylurea herbicides have high herbicidal potency, which significantly reduces the amount of the applied chemical to the field in comparison with conventional ones (Brown, 1990). However, the application of low levels of these herbicides does not guarantee high levels of environmental tolerance. In some cases, sensitive plants suffer damages by dosages lower than 1% of the initial application rate (Beyer et al., 1987). For most herbicides, the content of

soil organic matter and soil texture are the major factors affecting their phytotoxicity and persistence in the soil (Rahman et al., 2011).
High mobility of sulfonylurea herbicides in the soil environment (K_{oc}<100 ml/g), weak acid nature, pKa values ranging from 3.3 to 5.2 (Brown, 1990; Hay, 1990) and high value of GUS index, classify them into the herbicide with a potential phytotoxic action. Stability of these residual herbicides and their metabolites can affect sensitive plants species negatively during crop rotations. Sorption of sulfonylureas is low in all soils and they persist long enough in the environment to be ranked as leaches in all soils ($t_{1/2}$≥16.5 d). They are degraded in soil as a result of both microbiological and chemical processes, with the latter being particularly important in acid soils (Brown, 1990). Sulfonylurea herbicides, such as nicosulfuron, are more mobile in alkaline soils and in soils with lower organic matter content. The most applicable sulfonylurea herbicide in corn for successful control of annual and perennial weeds is nicosulfuron (Table 1).

Table 1.Nicosulfuron

IUPAC name	Structural formula	Molecular formula	Molecular mass	p*Ka*
2-[(4,6-dimethoxypyrimidine-2-ylcarbamoyl)sulfamoyl]-N,N-dimethylnicotinamide		$C_{15}H_{18}N_6O_6S$	410.4 g/mol	4.6 (25°C)

At the request of the European Commission, EFSA Panel on Plant Protection Products and their Residues developed guidance on how to derive the half-life for degradation in the top 30 cm of soil at reference temperature and moisture conditions. This half-life is an important input parameter in model simulations of the exposure of organisms in soil for annual crops under conventional and reduced tillage, and therefore this guidance is an important part of this revised methodology. They recommend the evaluation of field dissipation studies taking to represent degradation in the soil matrix from the soil surface, into account for estimating half-life (EFSA, 2012). The studies performed in the last 30 years showed that about 90% of the applied herbicide rates degrade during the season in which they were used. However, the large differences in the persistence of herbicides during the season had temperature, moisture and soil pH. This is especially important when the following crops in crop rotation are legumes that may be susceptible to sulfonylurea herbicide residues present in an amount of only 1% of the applied quantity (Ferris, 1993). The phytotoxic effect of nicosulfuron and its soil metabolites on dicotyledonous plants leads to a self-limitation in the re-planting period. After sowing, in 27-30 days interval since pesticide application, phytotoxic effects are obvious on cereals, sugar beet, canola and clover. The listed crops should not be sown on land previously treated by nicosulfuron before next spring, when the expected level of herbicide residues and their degradation products is <0.001 mg/kg.
For those reasons, numerous tools have been developed to predict the relative persistence and risk of damage from these herbicide residues. In this study, field experiments were designed to investigate the dissipation dynamics and final residues of nicosulfuron in soil. Modified QuEChERS procedures and high performance liquid chromatography (HPLC)-UV detection method were used to detect nicosulfuron residues. This study provides the basis to assess environmental risks. These results would be significant in providing guidance on the proper and safe use of nicosulfuron in field application.

Material and methods

Field experiment design;

The experiment conducted in this research includes the dissipation study and final residues study. The supervised field trial was carried out on a chernozem soil, in the surrounding of Novi Sad. Field trial was conducted according to OEPP standard methods for experimental design and data analysis. In the corn field, nicosulfuron (OD, 40 g/l) was applied at the recommended dose of 44 g/l. The nicosulfuron was sprayed post-emergence at the corn growth stage of BBCH 12-18. Soil samples were collected before and immediately after the pesticide application, and every two weeks till harvest, from surface soil layer, of 0-30 cm.

Chemicals and solutions;

The analytical standard of nicosulfuron (97%, purity) was purchased from Dr Ehrenstorfer (Augsburg, Germany). Nicosulfuron 40 g/l oil-dispersion (OD) was purchased from Galenika Fitofarmacija AD. Acetonitrile (ACN, HPLC grade), formic acid and acetic acid purchased from J.T. Baker (Germany). Purified water was prepared by using Milli-Q water purification system (TKA, Germany). For the soil sample extraction, a ready-to-use QuEChERS dispersive SP extraction (Cat. No. 5982-5650) kit was purchased (Agilent Technologies, USA). Nicosulfuron stock standard solution (1000 µg/mL) was prepared in acetonitrile (HPLC-grade) and stored at -20 °C. The working standard solutions (0.5–10.0 µg/mL) for calibration and quantitation were prepared immediately before analysis through dilution with acetonitrile.

Chromatographic Parameters;

Chromatographic analysis and extraction procedure were described in our previous publication (Lazić et al., 2014). For the chromatographic analysis, an Agilent 1100 Series system with DAD detector and Zorbax SB-C18 column (5 µm, 250mm ×3 mm internal diameter) were used. The mobile phases, which were composed of acetonitrile (A) and 0.1% (V/V) acetic acid in water (B), were pumped at a flow rate of 1.0 ml/min. The following gradient profile was used: 0–10 min linear from 52% to 47% (A). The external standard and calibration on five levels were used.

Extraction Procedure;

Soil samples were dried at room temperature, milled and sieved. After that, 10.0 g of soil sample was weighted into polypropylene tube of 50 ml volume, 3 ml of deionized water and 10 ml of acidified acetonitrile were added. The tube was shaken and vortexed for a 1 min. A mix of buffered salts from separate pouches was added, shaken for 1 min and vortexed 1 min. The tube was placed in an ultrasonic bath for 10 min and centrifuged at 4000 rpm for 5 min. The supernatant was filtered through a 0.45 µm membrane filter and transferred into an autosampler vial for HPLC-DAD analyses.

Results and discussion

The results for determination of nicosulfuron in trace amount were presented in the paper of Lazić et al., 2014. The validation study was performed according to SANCO/825/00 rev. 8.1 16/11/2010. Satisfactory linearity was obtained when the correlation coefficient (r^2) was 0.9999, based on measurement of the analyte peak areas in the soil. The limit of detection LOD and limit of quantification LOQ for nicosulfuron in soil were 0.01 and 0.05 mg/kg respectively. Recovery studies were performed, to validate the HPLC method, by spiking the blank samples at two concentration levels (0.05 mg/kg and 1.0 mg/kg) showed satisfactory result with average recovery of 83.9%, with the relative standard deviation of 1.5%. The precision of the method was 1.83%.

As shown in Figure 1, the results of dissipation demonstrated a gradual and continuous decrease of nicosulfuron content in soil. The initial deposit of nicosulfuron in soil was 0.95

mg/kg. Herbicide concentration decreased over time to 0.59, 0.29 and 0.12 mg/kg in five months period.
The dissipation curve of nicosulfuron in soil was fitted with first order kinetics and the degradation rate constant. Half-life was calculated using the equation (1)

$C_t=C_0e^{kt}$ (1)

where C_t represents the concentration of the pesticide residue at the time of t, C_0 represents the initial concentration after application and k is the dissipation degradation rate constant (days). The half-life of nicosulfuron in soil was calculated from the k value, $t_{1/2}$=ln2/k. The dissipation equation for nicosulfuron, achieved in this experiment was $C=0.839e^{-0.10x}$, with the half-life of 6.93 days (Table 2).

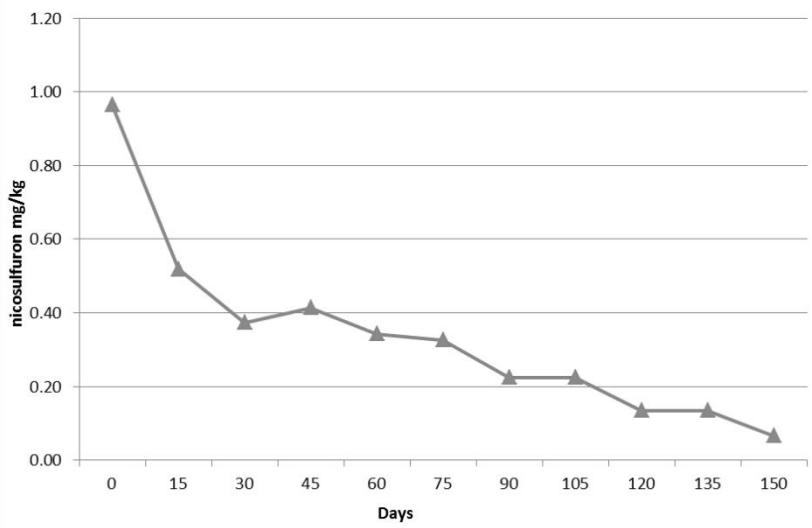


Figure 1. Dissipation curve of nicosulfuron in soil

Table 2. Half-life and data parameters for nicosulfuron dissipation in soil

Sample	Regression equation	Degradation constant (days)	Determination coefficient (R^2)	Half-life (days)
Soil	$y=0.839e^{-0.10x}$	0.10	0.864	6.93

Acetolactate synthase inhibitors (ALS) herbicides have been shown to vary widely in their degradation rates in response to soil pH, organic matter content and temperature (James et al. 1999). The cation-exchange capacity, principally a function of clay type and organic-matter content, is directly involved in herbicide adsorption. The content of organic matter in chernozem soil type, on which the trial was set up, determined by the method of wet incineration (Simakov, 1957) was 3.05-3.47% (Ćirić et al., 2013). With the increase of organic matter content, nicosulfuron adsorption also increases. Herbicide molecules from the soil solution are removed by adsorption, therefore decreasing its phytotoxicity but also making it unavailable for degradation (Loux and Reese, 1993). In this research pH of the soil is also evaluated. pH value of 7.54, indicates weakly alkaline soil. Generally, the rate of degradation of sulfonylurea herbicides in soil is fastest in warm, moist, light-texture and low pH soils, and slowest in cold, dry, heavy and high pH soils (Rao, 2000). It appeared that soil

organic matter followed by soil pH had the greatest effect in reducing herbicide residue phytotoxicity in the soil.

Ashburn et al., 2002 established that nicosulfuron half-life in soils is 5.3 and 4.2 days, when it is applied alone and in a mixture with rimsulfuron. Studies of Azcarate et al. 2015, showed that half-life of nicosulfuron in surface soil layers were 5-7 days. On the other hand, reported DT_{50} values for nicosulfuron in soil were much longer, from 14 to 49 days (Russell et al., 2002). According to EFSA, DT_{90} values of nicosulfuron range between 30 and 210 days.

The dissipation of nicosulfuron in soil in our experiment was slightly faster than that in some of previously conducted researches. It could be the consequence of extreme weather conditions in days after herbicide application. In the region on which the trial was set up during May, in days after the treatment there was more than 200 mm of precipitation, while annually fell the total 821.2 mm of rain. Such conditions could cause intensive nicosulfuron leaching into deeper soil layers, i.e. low herbicide retention in the surface layer. However, as it was mentioned before, half-life is only a rough indication of the potential persistence of herbicide active ingredients in soil (Sekutowski, 2011). In field conditions, herbicide degradation and translocation are slower or more intensive, depending upon the physical and chemical properties of the compound and their interaction with environmental conditions. In such a manner, herbicide persistence and translocation in soil cannot be considered only by one of the named parameters (e.g. $t_{1/2}$, Koc, Rf), but numerous environmental factors are to be included to provide information on herbicide fate in the environment. For the prediction of the mobility of pesticides in the field, the most used index is the Groundwater Ubiquity Score (GUS), (Gustafson, 1989). GUS index is based on the values of $K_{oc,}$ sorption coefficient normalized to soil organic carbon content and on a half-life. According to the literature data, GUS index for nicosulfuron is 3.34, which classifies it into the compounds with high potential of leaching (Pfeiffer, 2010).

Conclusions

In this paper the dissipation rate and residues of nicosulfuron in soil under field conditions were investigated. The final residues of nicosulfuron were ranged from 0.96 to 0.07 mg/kg and nicosulfuron half-life in soil was 6.93 days. Results of the field trial showed that soil properties, as well as climatic conditions have significant influence on nicosulfuron presence in the soil during the season in which it has been applied.

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CONTROL OF DIABROTICA VIRGIFERA VIRGIFERA LE CONTE WITH INSECTICIDES

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Abstract

Diabrotica virgifera virgifera Le Conte is a significant, introduced harmful species in maize in production conditions of northern Serbia. The aim of this study was to investigate the efficacy of indoxacarb (150 g a.i./l) 0.25 l/ha and bifenthrin (100 g a.i./l) 0.3 l/ha for the control of *D. virgifera virgifera*. The experiments were carried out in 2014 in Parage and Vilovo (Serbia, Vojvodina) according to standard EPPO methods (PP1 / 152; 1/135 and 1/274). Four assessments were made: before the treatment, three, seven and 15 days after the treatment by counting adults on 30 plants/replication. The efficacy (E%) was calculated according to Henderson-Tilton. In Parage the average number of adults of *D. virgifera virgifera* before the treatment was 33-44. Three days after the application of insecticides the number of adults (0 to 2.25) was significantly lower compared to the control (21.5) and insecticide efficacy was 92.5 to 100%, depending on the insecticide. Also the number of adults (0.75 to 4.75) after seven and 15 days (1.75 to 2.75) was significantly lower compared to the control (18.5 to 24.5) and the efficacy ranged from 85.4 to 97.3% i.e. 87.1 to 92.9%, respectively. In Vilovo, before the application of insecticides the number of adults was 24.5-27.5, while three days after (2.5 to 3.75) was significantly lower compared to the control (27.5) and insecticides efficacy was 84.6 to 90%. After seven days the number (1-1.5) was significantly lower compared to the control (24.7) and insecticides efficacy was 93.1 to 95.5%. After 15 days adults of *D. virgifera virgifera* were not recorded in treatments with insecticides.

Keywords: maize, *Diabrotica virgifera virgifera*, indoxacarb, bifenthrin, efficacy

Introduction

Diabrotica virgifera virgifera Le Conte is a significant, introduced pest of maize in production conditions in northern Serbia. The spread of this pest species is favored by the fact that about 40% of arable land is under maize, that the land after property transformation belongs to non-professionals and that the scientific contribution of crop protection was ignored so the areas under maize in monoculture are rapidly increasing. In Serbia, repeat sowing of maize is present in about 30% of the total area and even over 80% in some regions (Stanković et al., 1998). In the 90`s, in Serbia maize was grown in monoculture on about 40% of the state land and about 50% in the private sector, which favored this newly introduced pest and contributed to its rapid expansion in the mentioned area (the collective of authors, 1995). Given the above mentioned, in recent years chemical control is necessary measure in *D. v. virgifera* control. For the control of this pest in Serbia only preparations for seed treatment on the basis of imidacloprid and clothianidin were registered. However, due to problems arising around the intoxication of bees and loss of honey bee colonies, which is attributed to neonicotinoid insecticides, in a number of European and other countries, certain restrictive measures (EU Regulation 485/2013) have been undertaken. Ministry of Agriculture and Environmental Protection of the Republic of Serbia, has also introduced a moratorium on the use of preparations based on imidacloprid and clothianidin for maize seed treatment, so now for the control of *D. v. virgifera* larvae there is only one registered insecticide for seed

treatment –a.i. tefluthrin. For the control of adult *D. v. virgifera* in foliar treatments, for now, there is not a single insecticide registered in Serbia (Savčić-Petrić, 2015).
The aim of this study was to investigate the possibility of adult *D. v. virgifera* control in maize production conditions in Vojvodina, given that there is not a single registered insecticide for this purpose in the Republic of Serbia.

Materials and methods

Experiments were conducted in the localities Parage and Vilovo (at both locations maize was cultivated in monoculture for two consecutive years with the problem of *D. v. virgifera* presence) using standard OEPP methods for experimental design and analysis (Anonymous, 2006), for phytotoxicity (Anonymous, 2006a) and the efficiency of insecticides in the control of adult *D. v. virgifera* (Anonymous, 2011). Sowing of hybrid Pioneer 9911 was conducted on 30.03.2014 in Vilovo, while the sowing of hybrid Pioneer 0412 AQUAMAX was on 08.04.2014 in Parage. Hybrid Aquamax 0412 is a new hybrid of the group 500 and a hybrid Pioneer 9911 is in maturity group 400. Both hybrids have similar characteristics. The stalk is higher with higher position of ears. Kernel is a hard dent type. As all hybrids and from this group, both are adapted to the regions with water deficit and are highly tolerant to prolonged high temperature.
Size of the basic experimental plot was 2500m^2, set in four replications, as randomized block design. In Parage treatment was carried out on 06/07/2014, while in Vilovo on 21.07.2014. Maize was at the stage BBCH 63-65 (beginning of the pollen rejection, visible silk threads, to the upper and lower parts of tassel in flowering, silk fully developed). Preparation Avaunt 15 EC (indoxacarb 150 g / l) was applied at rate 0.25 l / ha, and Fobos-EC (bifenthrin 100 g / l) at 0.3 l / ha, as foliar spraying using tractor sprayer (tractor with a high clearance). The number of adult *D. v. virgifera* was monitored on yellow sticky traps, 3 days before the experiment, as well as after treatment. Efficacy evaluation was performed by monitoring the number of adults on 30 plants (in three places on 10 consecutive randomly chosen plants) per replication. In Parage evaluations were made before the treatment (07.06.2014.), three days after (09.07.2014.), seven days after (07.13.2014.) and 15 days after the treatment (07.21.2014.). In Vilovo, evaluations were made before the treatment (21.07.2014.), two days after (23.07.2014.), seven days after (28.07.2014.) and 16 days after treatment (06.08.2014.). The efficiency (E%) was determined according to the Henderson-Tilton-in (Wentzel, 1963) and the significance in differences between the number of adults i treatments and the control was tested using ANOVA.

Results and discussion

Results on the control of *D. v.virgifera* adults in Parage and Vilovo are presented in Tables 1-5. Table 1 shows the number of adults on yellow sticky traps 3 days before the experiment, as well as after the treatment. Based on the presented results, it can be concluded that the number of adult insects decreased after the treatment with insecticides compared to the control.

Table 1. The number of *D. v.virgifera* adults caught on yellow sticky traps

Parage				Vilovo			
Date	No of imagoes			Date	No of imagoes		
	I	II	III		I	II	III
03.07.2014.	7	10	12	18.07.2014.	6	6	7
04.07.2014.	8	13	14	19.07.2014.	5	8	9
05.07.2014.	14	9	8	20.07.2014.	9	11	8
06.07.2014.	11	15	12	21.07.2014.	7	8	6
09.07.2014.	0	0	9	23.07.2014.	0	0	11
13.07.2014.	1	2	11	28.07.2014.	0	1	10
21.07.2014.	2	2	10	06.08.2014.	1	1	6

I – Tape in a plot with insecticide Avaunt 15-EC; II – tape in a plot with insecticide Fobos-EC; III – tape in the control

Based on the observations before the experiment (Table 2) the average number of adults in Parage was 33.0 to 44.0 per replication. Strikingly, adults were present in a high number, but exceptional mobility does not give a true picture of the real numbers per plant. Three days after the application of Avaunt 15 EC (0.25 l / ha) and Fobos-EC, the number of adults was at a significantly lower level compared to the control (Table 1). The efficacy of the tested preparations three days after the application was 92.5 to 100%.

Table 2. Number of *D.v. virgifera* adults on maize plants before the setting of experiment and three days after and the efficacy in Parage

Insecticide (l/ha)	Before the treatment (06.07. 2014.)		Three days after treatment (09.07.2014.)		
	x	Sd ±	x	Sd ±	E %
Avaunt 15 EC (0.25)	**44.0**	10.39	**2.25 b**	1.5	**92.5**
Fobos-EC (0.3)	**38.0**	10.48	**0 b**	0	**100**
Control	**33.0**	3.74	**21.5 a**	7.05	
LSD 5%			7.85		

x-average number; Sd – standard deviation; E%-efficacy

Seven days after the application of insecticides, the number of adults was on significantly lower level in comparison to the control (Table 3). Efficacy of the tested preparations for the control of adult *D.v. virgifera* ranged from 85.4 to 97.3%. The number of the adults, 15 days after the application of Avaunt 15 EC and Fobos-EC, was on significantly lower level compared to control. Efficacy of insecticides ranged from 87.1 to 92.9%.

Table 3. Number of *D.v. virgifera* adults on maize plants seven and 15 days after the treatment and the efficacy in Parage

Insecticide (l/ha)	Seven days after the treatment (13.07.2014.)			15 days after the treatment (21.07.2014.)		
	x	Sd ±	E %	x	Sd ±	E %
Avaunt 15 EC (0,25)	**4.75 b**	2.75	**85.4**	**1.75 b**	1.25	**92.9**
Fobos-EC (0,3)	**0.75 b**	0.49	**97.3**	**2.75 b**	0.96	**87.1**
Control	**24.5 a**	7.59		**18.5 a**	3.42	
LSD 5%	8.05			3.77		

x-average number; Sd – standard deviation; E%-efficacy

The average number of *D.v. virgifera* adults in Vilovo prior to treatment was 24.5 to 27.7 per replication (Table 4). In this locality in visual assessment, the number of insects was slightly lower but in neighboring plots (outside the experiment) was much higher. Two days after the treatment with insecticides, the number of adults was significantly lower compared to the control. The efficacy of Avaunt 15 EC was 90% and of Fobos-EC 84.6%.

Table 4. Number of *D.v. virgifera* adults on maize plants before the setting of experiment and two days after the treatment and the efficacy in Vilovo

Insecticide (l/ha)	Before the treatment (21.07.2014.)		Two days after the treatment (23.07.2014.)		
	x	Sd ±	x	Sd ±	E %
Avaunt 15 EC (0.25)	**25.2**	5.62	**2.5 b**	1.73	**90.0**
Fobos-EC (0.3)	**24.5**	4.51	**3.75 b**	2.63	**84.6**
Control	**27.7**	2.50	**27.5 a**	2.38	
LSD 5%			2.98		

x-average number; Sd – standard deviation; E%-efficacy

Seven days after the treatment with insecticides, the number of adults was at significantly lower level compared to the control (Table 5). The efficacy of the tested preparations was 93.1 to 95.5%. 16 days after the application of insecticides, the number of adults was also significantly lower compared to the control. The efficacy of insecticides was 100%.

Table 5. Number of *D.v. virgifera* adults on maize plants seven and 16 days after the treatment and the efficacy in Vilovo

Insecticide (l/ha)	Seven days after the treatment (28.07.2014.)			16 days after the treatment (06.08.2014.)		
	x	Sd ±	E %	x	Sd ±	E %
Avaunt 15 EC (0.25)	**1.0 b**	0.81	**95.5**	**0 b**	0	**100**
Fobos-EC (0.3)	**1.5 b**	1.29	**93.1**	**0 b**	0	**100**
Control	**24.75 a**	3.09		**6.75 a**	2.22	
LSD 5%	3.47			2.21		

x-average number; Sd – standard deviation; E%-efficacy

In order to avoid the emergence of resistant strains of *D. v. virgifera* caused by frequent use of insecticides with the same mode of action, the use of tank mix combinations or alternative use of the preparation of different modes of action is recommended and is the main strategic principle in combating the resistance. For the control of *D. v. virgifera* no data on resistance to indoxacarb and bifenthrin exist. There is a certain level of resistance of *Helicoverpa armigera* Hübner towards indoxacarb (Pakistan) and in populations that are highly resistant to the insecticide belonging to the group of pyrethroids, organophosphates and carbamates (Ahmad et al., 2003).

Test results on the European corn borer indicate the nature of indoxacarb as pro-insecticides which requires hydrolytic activation (Analysis et al., 2008). By topically applying indoxacarb its toxicity was antagonized if the larvae were previously treated with DEF - an inhibitor of hydrolytic enzymes. DEF also inhibited the activity of hydrolytic enzymes and the formation of active metabolites, both *in vivo* and *in vitro*. Hydrolytic activation of indoxacarb is a unique among chemical insecticides and may be particularly important in the control of insects that have developed a resistance as a consequence of increased hydrolytic metabolism. If the same hydrolytic enzymes are responsible for the resistance to pyrethroids, carbamates and organophosphates in connection with activation of the indoxacarb, there is the possibility of negative cross-resistance to indoxacarb. Agricultural extension services pay a lot of attention

to detecting the presence of *D. v. virgifera* in Serbia, monitoring the conditions for the development, the occurrence and prognosis of this pest. However, one of the aggravating moments in the monitoring and prognosis is a long season of maize growing, with maize hybrids from early maturing to late once. The forecast and severity of larvae and adults depends on the soil type (normal or heavy soils), growing conditions (repeated sowing or monoculture), assortment (mercantile, seed, silage), as well as on the choice of the method for determining the critical number. But according to some authors also will depend on the abundance in the previous year, the sex ratio in the population and assembly plants (Edwards et al., 1994). Bearing in mind the above mentioned it is necessary to determine the economic thresholds and recommend adequate measures according to the conditions and good knowledge of pest biology.

Conclusions

Based on the tests and the results achieved in control western corn rootworm (*Diabrotica virgifera virgifear*) on commercial corn with insecticides Avaunt 15 EC (indoxacarb) and Fobos-EC (bifenthrin), the following conclusions can be made:

Three days after application of the products Avaunt 15 EC (0.25 l/ha) and Fobos-EC at Parage site, the insecticides showed efficacy of 92.5-100%, while in the locality Vilovo two days after the application of insecticide efficacy was 84, 6-90%.

Seven days after insecticides treatment, the number of western corn rootworm adults was significantly lower compared to the control at both locations. The efficiency of the tested products for control of adult corn rootworm ranged from 85.4-97.3%, depending on insecticide sand locality.

The number of *D. virgifera virgifera* adults 15, or 16 days after the application of insecticides was considerably lower level compared to the control in both locations. Efficacy of insecticides ranged from 87.1 to 100%.

The applied insecticides successfully controlled *Diabrotica virgifera virgifera* in corn.

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THE IMPACT OF COLLETOTRICHUM DESTRUCTIVUM ON RESISTANCE IN DIFFERENT RED CLOVER CULTIVARS

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Abstract

Colletotrichum destructivum O`Gara, inducer of alfalfa and red clover anthracnose, is widespread in many areas in Serbia. Considering that and, also, the damage it can cause, anthracnose is economically very important disease. During ten years period, especially in summer and autumn, plants with anthracnose symptoms were studied in alfalfa and red clover fields. Stem infection results in wilting and death of the upper portion of the steam, giving rise to the characteristic “shepherd’s crook“ symptom. Strain of *C. destructivum* were isolated from diseased alfalfa stems collected from different locations in Serbia.

To examine the level of sensitivity of different red clover genotypes in experimental conditions, one isolate Coll-8 (*C. destructivum* originating from Serbia) were used. Fourteen commercial red clover genotypes of different origin (K-27, K-39, K-32, K-38, Una and Kolubara originating from Serbia, Čiroku, Lea, Virgiana, Valentine, Wilo, Repio and Diana originating from USA) were examined in this study. Infected plants showed typical symptoms of anthracnose. Necrotic lesions appeared on the plant stems and gentle bending of the top of the upper third of stems occurred on tested plants. Necrotic lesions further spread onto the whole plant and in some plants led to withering. Assessment of damage by pathogen inoculation was based on the scale of 0-5. All studied isolate caused symptoms of red clover anthracnose in the plants. Investigated genotypes showed contrasting resistances to *C. destructivum* isolate. The aim of this study was to determine sensitivity of commercial red genotype.

Key words: anthracnose, *Colletotrichum destructivum*, genotypes, red clover, sensitivity

Introduction

Red clover (*Trifolium pratense* L. var. *sativum* Sherb.) is one of the most important forage crops in Serbia. The fungus *Colletotrichum destructivum* O` Gara, which is causing anthracnose disease in red clover, is an important limiting factor for red clover growth, plant vigor and persistence (Boland and Brochu, 1989). During the summer and autumn, diseased plants start appear in the field. *C. destructivum* was isolated from alfalafa stem lesions typical of *C. trifolii* (Graham et al., 1976). Conidia form in the acervuli on stem lesions. Rainsplash or wind carry the conidia to the growing petioles and stems. The fungus grows down infected stems into the crown and taproot, causing killing of tissue, predisposition to winter injury, wilting or plant death. Nevertheless, soil infections of alfalfa by *C. destructivum* have caused considerable yield losses in North America **(**Boland and Brochu, 1989), Europe (Robotic and Klokocar-Smit, 1983), North Africa and South Africa (Koch et al., 1989). The host range of *C. destructivum* is wide and includes legumes such as *Phaseolus lathyroides* L., *Glycine max* (L.) Merr., *Trifolium* spp., *Lotus* spp., *Leucaena leucocephala* (Lam.) DeWit., *Melilotus albus* Desr., *Vigna unguiculata* (L.) Walp., *Coronilla varia* L., as well as tobacco (*Nicotiana tabacum* L.) and dodder (*Cuscuta* spp.) (Latunde-Dada et al., 1996).

The aim of this study was to determine sensitivity of commercial red clover genotypes to *C. destructivum* in Serbia, and possibilities for breeding the resistant red clover genotype.

Material and methods

The sensitivity of commercial red clover genotypes was tested in order to find potential sources of resistance to *C. destructivum*. Fourteen commercial red clover genotypes of different origin (K-27, K-39, K-32, K-38, Una and Kolubara originating from Serbia, Čiroku, Lea, Virgiana, Valentine, Wilo, Repio and Diana originating from USA) were examined in this study.

Isolate included this study were selected in the spring of 2010, based on morphological and molecular traits. The study included one isolate from Serbia Coll-8 identified as *C. destructivum*. The experiment was conducted in the greenhouse of the Institute for forage crops, Kruševac (Serbia). Red clover seeds were sown in plastic containers at a depth of 2 cm in sterile substrate. Conidial suspension was prepared from cultures of tested isolate, grown for ten days on PDA medium at 25° C in the dark. The concentration of spores was determined by hemocytometer by Thom and it was 4-6 x 10^{-4} spores/ml. Inoculated red clover plants were old 7 week and scarred prior to inoculation by cutting and sprayed with spore suspension. The experiment was conducted in ten repetitions, 10 plants per genotype and red clover plants inoculated with sterile water used as a negative control.

Sensitivity of different red clover genotypes to isolates of *C. destructivum* was evaluated four weeks after inoculation using a modified method by Ostazeski et al. (1969), according to a scale of 1-5:

1 - no stem lesions or only few small water-soaked or black spots
2 - stems with elongated black lesions but without acervuli
3 - stems with long, wide, but non-gridling lesions, with acervuli present
4 - large, coalescing and sporulating lesions which gridle and kill upper part of seedling
5 - seedling dead

Based on the results, severity index was calculated according to the following formula:

$$I = \Sigma \frac{(n \times k)}{N \times K} \times 100$$

I = severity index	N = total number of seedling
n = number of seedlings in class	K = number of categories
k = number of individual categories	$\sum$ = sum of products

According Ostazeski et al. (1969), where 1 and 2 are assigned to resistant plants. Plants were scored individually, two weeks after inoculation. Disease intensity or severity of infected plants was calculated using the severity index.

Results and discussion

All studied isolate caused symptoms of red clover anthracnose in the plants. Four weeks after inoculation, necrotic spots in the lower parts of stem appeared in infected red clover plants of all genotypes (K-27, K-39, K-32, K-38, Una, Kolubara, Čiroku, Lea, Virgiana, Valentine, Wilo, Repio and Diana) (Table 1). As the disease progresses stem changed color to glossy yellow, the crown bended in the form of a hook and plants started to wilt.

According to Ostazeski et al. (1969), sensitive genotypes have 10% of healthy plants, while the genotypes with over 65% can be deemed resistant. Genotypes Arc, which is resistant to race 1 of *C. trifolii,* has the severity index 2.72 to 2.25 (Ostazeski et al., 1979). The isolate Coll-8 showed higher pathogenicity and number of resistant plant were very low in genotypes Kolubara and Wilo inoculated with this isolate (5%). By the severity index (3.3), it can be concluded that the genotypes Kolubara had the moderate resistance. Also genotypes Wilo showed a higher resistant to isolate Coll-8, with severity index 1.9.

The genotypes K-27 and Čiroku had the highest ratio of healthy plants (40%), but genotypes

K-27 had the severity index (3.2), this genotypes is moderate resistant to tested isolate. By the severity index (2.4), it can be concluded that the genotypes Čiroku is resistant to the studied isolate.

Table 1. Evaluation of sensitivity in commercial genotypes of red clover to isolate *C. destructivum* (Coll-8)

Genotypes	Percentage of plants in score classes					Severity index (%)	Resistant plants (1+2%)
	1	2	3	4	5		
K-27	0	40	10	0	0	3.2	40
K-39	0	10	25	10	5	3.2	10
K-32	0	10	20	10	10	3.4	10
K-38	0	35	5	20	0	2.5	35
Una	0	35	15	0	0	2.3	35
Kolubara	0	5	30	10	5	3.3	5
Čiroku	0	40	5	0	5	2.4	40
Lea	15	10	25	0	0	2.2	25
Virgiana	0	10	35	0	0	2.7	10
Wilo	0	5	15	10	0	1.9	5
Repio	5	30	10	5	0	2.3	35
Diana	0	15	20	5	10	3.3	15
Valentina	0	0	35	0	10	3.6	0
Kontrol	-	-	-	-	-	-	-

The genotypes K-38, Una and Repio showed similar results for sensitivity on the tested isolate, and they showed higher resistance to tested isolate *C. destructivum*.
Also genotypes K-39, K-32 and Virgiana had very low the number of resistant plant and they showed moderate resistance (Table 1). The genotype Valentina had the lowest resistance to tested isolate.
The results indicate high variability of genotypes responses to isolate. In addition, the response of individual plants within genotypes to inoculation with pathogenic isolate was also very variable. A small number of individual plants have managed, despite clear symptoms of disease, to produce seeds. Progeny of these plants is an excellent source of resistance to the tested isolates of the pathogen and will be included in the next cycle of selection for this trait. Considering the results of Devine et al. (1971) who showed that after several cycles of selection the level of resistance can be increased, we believe that it is justified to continue investigations in this direction. Boland and Brochu (1989), O'Neill et al. (1989) and Schubiger et al. (2003), in their studies of pathogenicity, showed that the species *C. trifolii* is more infectious for alfalfa than *C. destructivum*. Boland and Brochu (1989) and O'Neill et al. (1989) reported that *C. destructivum* in an enclosed space showed a higher level of infectivity and the same level of infection as *C. trifolii* in field conditions.
Three races of *C. trifolii* (races 1, 2 and 3) have been described based on differential responses of alfalfa cultivars (Ariss and Rhodes, 2007) conferred by two separate dominant genes, designated *An1* and *An2*, are attributed to anthracnose resistance. *An1* confers resistance to race 1 and likely, race 4, whereas *An2* confers resistance to races 1 and 2. It is noteworthy that the race 3 of *C. trifolii* was reported 1982 (Allen et al., 1982), but this fungus was subsequently reclassified as *C. destructivum* (Yang et al., 2008, Ameline-Torregrosa et al., 2008). Despite the large number of studies, which indicated that resistance to anthracnose is controlled by major dominant or recessive genes and other resistance mechanisms, resistance to *C. trifolii* is not clear enough (Melotto et al., 2000; Schubiger et al., 2004). In the

studies by Schubiger et al. (2003), only two Mattenklee cultivars (Pavo and Merula) exhibited 50% resistance or more to *C. trifolii*. The other Swiss cultivars of the persistent Mattenklee type showed an intermediate level of resistance. Vasić et al. (2010b) reported that investigated cultivars and breed populations showed various resistances to different *C. trifolii* isolates. The resistance degree depends on both the isolates of *Colletotrichum trifolii* and red clover cultivars. Vasić et al. (2010a) showed that alfalfa clones had different resistance to *C. trifolii*. Comparison of American and Serbian cultivars showed the American alfalfa cultivars had the higest level of resistance (64.5% plant survival). Among the varieties listed in Serbia, their cultivars showed the uneven resistance levels, the percentage of surviving plants was 0-56.4% on average. The most susceptible tested cultivar was with a plant survival rate of 56.4%.
Selected red clover genotypes that showed higher levels of tolerance to the tested isolate will be further used in field trials in order to accurately assess the level of resistance. This will enable the separate genotypes to be used in the further process of selection and breeding of red clover to improve the level of resistance.

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DETERMINATION OF THE MATERNAL EFFECT OF RHYZOBIUS LOPHANTHAE BLAISDELL (COLEOPTERA: COCCINELLIDAE) BY USING LIFE TABLE

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Abstract

Citrus is one of the most important cultivated crops in Turkey. As for all agricultural crops, plant protection problems such as pests and diseases are the major factors decreasing crop yield. There are more than 150 pest species causing damage on citrus. Among them scale insects are main pests. There are lots of predators and parasitoids feeding on scale insects. *Rhyzobius lophanthae* Blaisdell, a polyphagus predator, is one of the most important predators of scale insects and widely distributed in citrus plantations in Turkey.

In this study, we investigated the maternal effect on the reproduction and development of *R. lophanthae* at the 1st, 10th, 20th and 30th days. One day age adults are used in the treatments. *Aspidiotus nerii* was used as the prey. Trials were performed in a climatic chamber with 26±1 °C temperature and 65±1 % RH.

The average numbers of offsprings produced by the eggs laid by 1, 10, 20 and 30 days old females were 179.0, 143.0, 264.0 and 314.5, respectively. Life table parameters were also calculated. Net reproductive rate ($\mathbf{R_0}$), intirinsic rate of increase ($\mathbf{r_m}$) and mean generation time ($\mathbf{T_0}$) were found to be 24.409, 19.067, 24.256 and 25.732 females/females/generation, 0.130, 0.107, 0.132 and 0.106 females/females/day, 24.658, 27.456, 24.227 and 30.671 days, respectively.

Keywords: Maternal effect, *Rhyzobius lophanthae*, intrinsic rate of increase, life table

Introduction

Citrus is the most important cultivated crop among fruits in the World. Turkey has an important place in terms of citrus production among the Mediterranean countries (Uzun, 2012). The Mediterranean Region of our country is the most important cultivation area for citrus production. In recent years, it was reported that there had been a significant increase both in the number of pests and the number of populations in this area (Uygun and Karaca, 1998). Among these pests, Diaspididae which are called scale insects are very important and one of the most important species is Red scale insect *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) (Kansu and Uygun, 1980). Although measures are taken against these pests, they can destroy citrus trees in a short time (Anonymous, 1991; 2008).

Various control methods have been applied against these pests that cause major damage on citrus. The most common method is chemical control. However, populations of the pests increase in a short time after the applications of insecticides and they become harmful again (Uygun and Şekeroğlu, 1981). So, it seems that chemical control is a temporary solution. In addition, pesticides disturb the natural balance and have given rise to undesirable chemical drug residues on the products. Nowadays, studies have continued on the development of integrated pest management (IPM) which aims to keep pest populations below economically damaging level without adversely affecting beneficial insects in agriculture. ‘‘Biological Control’’ is the most important method in IPM (DeBach, 1969; Uygun et al., 1987).

The majority of the agents used in bological control are insects, and the majority of the insects are from Coccinellidae. *Rhyzobius lophanthae* Blaisdell (Coleoptera) which belongs to this

family is known as an important predator of scale insects (Hodek, 1973; Hodek ve Honek, 1996). In the studies carried out in our country, it was reported that this predator was common in citrus areas where scale insects were intensive (Tunçyürek, 1970; Öncüer, 1977; Kansu and Uygun, 1973; 1980; Uygun and Şekeroğlu, 1981; Uygun et al., 1987; Uygun vd., 1992 a, b; Uygun vd., 1995).

In this study, we investigated the maternal effect on the reproduction and development of *R. lophanthae* at the 1st, 10th, 20th and 30th days.

Materials and Methods

Aspidiotus nerii culture:

In this study, *A. nerii* population which was reared on potato tubers in the insectarium of Biological Control Laboratory of the Department of Plant Protection, Faculty of Agriculture, Süleyman Demirel University, was used. Clean potato tubers in plastic trays were deliberately infested with crawlers of *A. nerii* to build up the population. This procedure was continued for the duration of the study for two or three times a week. Rearing studies of *A. nerii* were carried out in rooms adjusted to 25±1°C, L:D 16:8 h photoperiod and 65±5% RH.

Rhyzobius lophanthae culture:

In this study, individuals of *Rhyzobius lophanthae* which were used as main material were collected from the citrus areas intensively infested with the pest, in Antalya (Steiner, 1962). In this way, both adult and immature stages of this predator insect were separately dropped into the jar with potatoes infested by scale insects (Nar et al., 2009). Potatoes were changed at least twice per week, in the culture jar throughout the study. Rearing studies of *A. nerii* were carried out in rooms adjusted to 25±1°C, L:D 16:8 h photoperiod and 65±5% RH.

Establishment of the experiments:

In this study, 9x9x8 cm plastic storage containers were used for individuals of *Rhyzobius lophanthae*. Pupae of this insect which were obtained from production source were taken into the container. After they became adults, 20 individuals (10♀, 10♂) were taken into separate containers. Eggs, left by the females completed the development, were separetely transferred into trial containers (an egg onto one potato tuber). Four different experimental groups were formed from females in this population. Eggs laid in the 1st, 10th, 20th and 30th day were taken onto separate potatoes infested with the scale insect. Individuals, belonged to each experimental group were observed from egg to death. During this time, each development stage and the number of offsprings were daily recorded. At least 20 repetitions were used for each experimental group. Rearing studies of *A. nerii* were carried out in rearing rooms adjusted to 27.5±1°C, L:D 16:8 h photoperiod and 65±5% RH.

Life table, Weibull distribution and analyses

The data that was obtained at the end of the trial were evaluated with age-related life table for each experimental group. All parameters were calculated by RmStat-3 according to Euler-Lotka equality (Birch, 1948).

Parameters were:

Survival rate (l_x) and fecundity (m_x), (Birch, 1948),

Net reproductive rate, $R_0 = \sum l_x.m_x$ (Birch, 1948),

Intirinsic rate of increase (r_m), $\sum e^{(-r_m.x)}.l_x.m_x = 1$ (Birch, 1948),

Mean generation time, $T_0 = \frac{\ln . R_0}{r_m}$ (Birch, 1948),

Gross reproduction rate, $GRR = \sum m_x$ (Birch, 1948),

Finite rate of increase, $\lambda = e^{r_m}$ (Birch, 1948),

Doubling time, $T_2 = \frac{\ln 2}{r_m}$ (Kairo & Murphy, 1995)

Reproductin value, $V_x = \frac{\sum_{y=x} (e^{r_m \cdot y} . l_y . m_y)}{l_x . e^{-r_m . x}}$ (Imura, 1987),

Life expectancy, $E_x = \frac{\sum_{y=x} \frac{l_y + l_{y+1}}{2}}{l_x}$ (Southwood, 1978; Carey, 1993),

Stable age distribution, $C_x = \frac{l_x . e^{-r_m . x}}{\sum_{x=0} (l_x . e^{-r_m . x})}$ (Birch, 1948).

Life table parameters were calculated for the populations at different parantal ages. The differences in r_m values were tested for significance by estimating the variance using the jack-knife method (Meyer et al., 1986; Özgökçe & Atlıhan, 2004). Then, these values were used in the Tukey multiple comparison test. In this study, age-dependent survival curves of the populations of different ages were obtained by Weibull distribution (Pinder et al., 1978).

$$S_p(t) = e^{\left[-\left(\frac{t}{b}\right)^c\right]} \qquad t, b, c > 0$$ (Deevey, 1947).

In the above formula; $S_p(t)$ is probability of survival, b is measure of slope, c; form of slope, t is time. The values of C parameter could be c > 1, c = 1, c < 1. Adhering to these values, c > 1 represents growing populations, c = 1 represents stable populations and c < 1 represents decreasing populations (Deevey, 1947; Pinder et al., 1978). The parameters that were obtained according to this curve is calculated using CurveExpert pro (ver. 1.6.7), SPSS (ver. 17), MS Excel (ver. 2003).

Results and discussion

In this study, survival rate (l_x), fecundity (m_x) and reproduction value (V_x) of the different age groups are shown in the graphic. As seen in the graphic, survival rates decreased and fecundity (m_x) and reproduction value (V_x) demonstrated differences depending on the age (Figure 1).

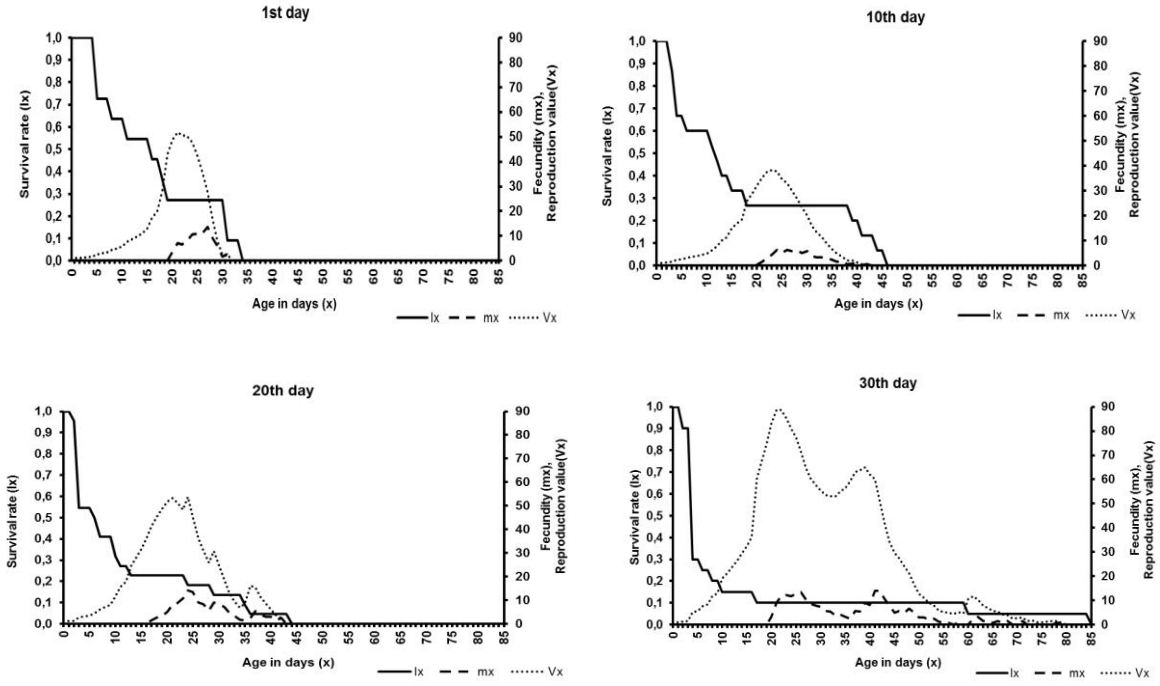

Figure 1. Survival rate (l_x), fecundity (m_x) and reproduction value (V_x) of *Rhyzobius lophanthae* in different age groups

Total development time and the other periods of *Rhyzobius lophanthae* in the different mother age groups were given in Table 1. Hatching times of the eggs were same for each age groups. Although there were differences between the development stages of larvae in different groups, pupae and adult stages were statistically same.
Preoviposition times were between 2.000 – 4.750 days, oviposition times were between 8,333 – 37,000 and postoviposition times were between 1,000 – 10,000 days in different groups (Table 1).

Table 1. Total development time and the other periods of *Rhyzobius lophanthae* in the different mother age groups

	1st day	10th day	20th day	30th day
Developments times and reproduction	**Mean ±St. err**	**Mean ±St. err**	**Mean ±St. err**	**Mean ±St. err**
Egg	4,375 ±0,183a	4,000±0,000a	4,083±0,193a	4,500±0,224a
Larvae1	2,750 ±0,250a	2,000±0,000b	1,900±0,100b	2,200±0,200ab
Larvae2	2,857 ±0,143a	2,111±0,111b	1,750±0,164b	1,667±0,333b
Larvae3	2,400 ±0,510a	2,125±0,125ab	1,286±0,184b	1,333±0,333ab
Larvae4	3,000 ±0,577b	2,750±0,250b	4,600±0,245a	5,000±0,000a
Pupae	3,667±0,333a	3,500±0,289a	3,800±0,200a	3,000±0,000a
Total development time	18,667±0,882a	16,500±0,289a	17,200±0,663a	17,000±0,000a
Preovipozition	3,667±0,333ab	4,750±0,629a	2,000±0,000b	3,500±0,500ab
Ovipozition	8,333±0,882b	17,500±1,708ab	13,000±4,147b	37,000±13,000a
Postovipozition	1,333±0,333b	3,000±0,000b	1,000±0,000b	10,000±5,000a
Adult life time	13,333±0,667b	26,000±1,683b	16,400±3,842b	55,500±12,500a
Total life time	16,636±3,350a	17,533±4,213a	11,864±2,747a	11,900±4,780a
Daily fecundity	13,401±0,781a	5,409±1,414a	10,303±3,736a	5,420±1,095a
Total fecundity	179,000±15,875a	143,000±38,074a	264,000±80,238a	314,500±128,500a

In this study, life table parameters of different groups were given in Table 2. As a result of the analyses, intrinsic rate of increase (r_m) was found between 0.106 - 0,132 female/female/day and there was not statistically significant difference between age groups. The values of net reproductive rate (R_o), mean generation time (T_0) and gross reproduction rate (**GRR**) showed differences among different groups (Table 2).

Table 2. Life table parameters of *Rhyzobius lophanthae* in different mother ages

	Mother age			
Life Table Parameters	**1st day**	**10th day**	**20th day**	**30th day**
Euler-Lotka (Birch, 1948)				
Intrinsic rate of increase, r_m	0,130a	0,107a	0,132a	0,106a
Net reprocution rate, R_o	24,409	19,067	24,256	25,732
Mean generation time, T_o	24,658	27,456	24,227	30,671
Gross reproduction rate, ***GRR***	91,167	72,333	149,283	266,318
Doubling time T_2	5,349	6,456	5,266	6,546
Finite rate of increase, λ	1,138	1,113	1,141	1,112
n	11	15	22	20

In the literature, it is reported that intrinsic rate of increase (r_m) of *R. lophanthae* was 0.122 female/female/day (Stathas et al., 2005) and between 0.038 – 0.155 female/female/day at different temperatures (Nar et al., 2009). Survival rates of adults of different groups were given in Table 2. According to the C parameter that determine the format of the slope of the Weibull distribution, all groups is similar to Type 3 ($c < 1$) of Holling. In addition, values of C parameters according to different groups were given in the Table 3 and graphics of Weibull distribution were given in Figure 2.

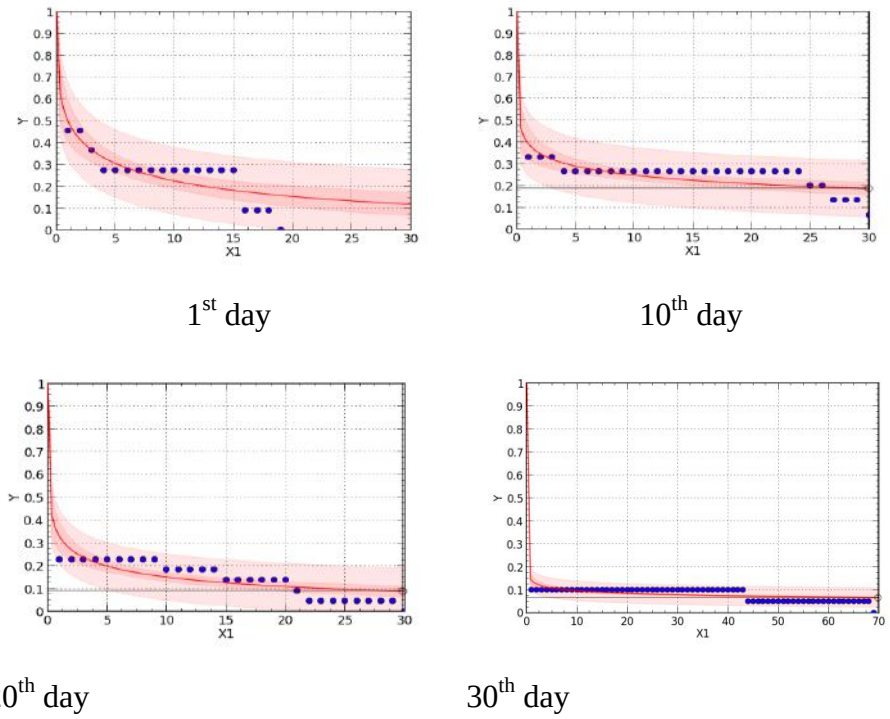

Figure 2. Survival rates of adults of different groups

Table 3. Values of C parameters according to different groups

Parameters	1st day	10th day	20th day	30th day
b	2.95±0.66	1.43±0.71	0.60±0.27	0.60±0.27
c	0.33±0.061	0.17±0.04	0.23±0.035	0.08±0.01
Type	3	3	3	3
R^2	0.65	0.41	0.61	0.61

In conclusion, *R. lophanthae* in the four different mother age groups show similar reproduction rate patterns. Thus, it can be concluded that parents developed from eggs laid by adults of different age could represent same reproductive performance. However, it is necessary to study reproduction behaviour of the natural enemy under field conditions.

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DETERMINATION STUDIES ON THE REACTIONS OF SOME WHEAT GENOTYPES FOR DRY LANDS AGAINST STRİPE RUST (Puccinia striiformis f. sp. tritici)

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Abstract

This research was conducted to determine the reaction of 25 (18 lines and 7 cultivars) bread wheat genotypes developed for dry lands against Stripe Rust (*Puccinia striiformis* f.sp. *tritici*) in 2012-2013 growing seasons in Konya ecological conditions. The inoculation of Stripe Rust was performed artificially. Observations and evaluations of Stripe Rust disease development were made according to Modified Cobb scale. 11 of 18 bread wheat lines showed immune level of resistance against Stripe Rust. No. 8 line was evaluated as the most susceptible genotype with 100S value. Among the standard cultivars, Bezostaja and Gerek were determined as very susceptible with 100S value. Regarding to results, candidate varieties namely, BDME 09/1K, BDME 11/1K, and BDME 11/2K, were evaluated as highly resistant to Stripe Rust.

Key words: *Bread Wheat, Dry areas, Stripe Rust, Genotype, Reaction*

Introduction

Wheat (*Triticum aestivum* L.) is the first important and strategic cereal crop for the majority of world's populations. Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman and Graur, 1995). Despite of its economic importance, wheat is attacked by number of pathogens which cause diseases like fungi (rusts, smuts, bunts etc.), bacteria (bacterial leaf blight, bacterial mosaic, black chaff etc.) and virus (wheat dwarf, wheat spot mosaic, wheat streak mosaic etc.). Food protection of the world, rely on production of two important cereal crops i.e., rice and wheat. Wheat rusts problem has emerged due to attack of fungus all over the wheat growing areas of the world.

Leaf rust caused *Puccinia recondite* f. sp. *tritici* and Stripe Rust caused *Puccinia striiformis* f. sp. *tritici* and stem rust caused by *Puccinia graminis* f. sp. *triticiare* potential risk that cause decrease in the yield of wheat (Singh et al., 2005). It has been known that the crop yield loss has ranged between %12-15 since yellow rust and unexpected disease epidemics can cause enormous yield losses (Kınacı, 1982). The rust diseases can occur in all wheat production areas; in general yellow rust is epidemical in Middle and East Anatolia, while stem and leaf rust are common in Aegean and Marmara Regions and in Southeastern, only stem rust is widespread in Turkey (Aktaş, 2001).

Stripe Rust (*Puccinia striiformis* f.sp. *tritici*) is an important disease threatening wheat production and it can be seen in majority of wheat growing area and from time to time its epidemics can cause significant loss in crop yield and quality. When the spring is cool and rainy through the years, the pathogen can multiply very fast and spread out by wind to long distances. According to research reports, in the years of 1991 (in Central Anatolia) and 1995 (in Çukurova Region), it was experienced significant crop yield loss due to yellow rust epidemics in Turkey (Braun and Saari, 1992; Düşünceli et al., 1999). As an alternative to chemicals which have many side effects such as being non-economic, disruption of the natural balance, environmental pollution, researchers focused on developing of new resistant

varieties for plant disease control. Since the pathogen can often develop new race, the continuous studies on developing new varieties has become necessary.
Therefore in this study, it was aimed to determine the reactions of some wheat lines developed in Bahri Dağdaş International Research Institute for dry lands against to Stripe Rust in 2012-2013 years.

Material and method

The plant materials were consisted of wheat lines at the stages of regional yield trials that were developed for dry lands by breeders at Bahri Dağdaş International Research Institute and the experiments were conducted in the growing season of 2012-2013 years.
The reactions of genotypes were tested in disease nursery of Bahri Dağdaş International Research Institute. The plantings were done with 1 meter rows and 5-7 depth one replication for each line/variety. As control plants, one row of Stripe Rust susceptible variety “Little Club” was planted every 5 rows. Additionally, the experimental area was surrounded with “Little Club” as six rows to encourage the disease development.
As inoculum source, Stripe Rust uredospores, which were collected from field a year in advance and stored in liquid nitrogen, were used. Inoculation was prepared as mixture of water, Tween 20, glycerol, and Stripe Rust uredospores. Sunset, rain, and cloudy weather were preferred for plant inoculations. Besides, mini sprinkle system was used for irrigation and encouraging the disease progress on dry days.
The evaluation of reactions of genotypes was done according to Modified Cobb Scale (Peterson et al., 1948) and the severity of stipe rust and infection types were recorded. When the disease development on control plants “Little Club” reached 90-100S scale value, the reactions of genotypes were evaluated twice through the season. The final disease severity data for the Stripe Rust was converted into a coefficient of infection (CI) by multiplying severity with a constant value for field response (Yadav, 1985; Stubbs et al., 1986; and Roelf et al., 1992). In order to calculate CI, for infection type values were S (Susceptible): 1, MS (Moderately Susceptible): 0.8, M (Moderately: intermediate reaction): 0.6, MR (Moderately Resistant): 0.4, R (Resistant): 0.2 (Stubbs et al., 1986). In the growing season of the 2012-2013years, the numbers of tested plant materials are given in Table 1.

Results and discussion

The disease development of Strip Rust was accomplished by artificial inoculations for the growing seasons in 2012-2013 years and the performance of each line/variety was determined. Evaluation was made when the disease score reached 90-100S on control plants “Little Club”. According to results, among 25 genotypes including lines and varieties, the Stripe Rust severity was varied between 0-100 percent. As seen in Table 1, the varieties of Gerek, Bezostaya, no.10, and no.11 genotypes were highly susceptible with 80-100S score values while genotypes numbered as 7, 8, 14, 15, 17, 18, 19, 20, 21, 22, and 23 were determined as resistant at immune level. No. 12 and 16 genotypes gave moderately resistant (MR) reaction with 10% disease severity while No.13 line and the variety of Tosunbey were moderately susceptible (MS) with %20 disease severity.

Table 1. The Disease Severity and Reaction Type of Lines/Varieties against Stripe Rust

Numbers of Lines/Varieties	Severity of Stripe Rust (%)*	Reaction**	Varieties/Lines
1	40	MS	**BAYRAKTAR**
2	100	S	**GEREK**
3	40	MS	**KARAHAN**
4	20	MS	**TOSUNBEY**
5	100	S	**BEZOSTAYA**

6	40	MS	ZENCİRCİ/BACANORA
7	0	R	BEZ4/4/LOV10/CD*2//CO//CO/3/SANTACATALINAKENYA2//4-11/5/RPB 8-68/CHRC/3/BEZ//BEZ/TVR
8	0	R	KIRGIZ95/YANTAL7578-128
9	40	MS	KOL/PMF/3/7C//CNO/CAL/4/CLEO/5/21031/TPR//CO652643/6/SONMEZ01
10	80	S	CHAM//1D13.1/MLT/4/C126-6/C190-12//AU/3/TZPP/BEZ
11	100	S	Karahan / Konya
12	10	MR	KRC/BEZ/3/1150-18/VGDWF/4/YE2453/5/BEZ/NAD//KZM (ES85.24)/3/F900K
13	20	MS	GV/4/D6301/NAİ//WRM/3/CNO*3/ CHR/5/ BL2973/6/ LOVRİN6/SAMSUN
14	0	R	OK81306/MERCAN-2
15	0	R	SEAFALLH/GUN91/7/NE COMP1/5/BEZ//TOB/8156/4/ON/3/TH*6/KF//LEE*6/K/6/TAST/SPRW
16	10	MR	NE COMP1/5/BEZ//TOB/815/6/AGRI/BJY//VEE
17	0	R	BEZ/NAD//KZM (ES85.24)/3/F900K/4/AEG TAUSHİ/CHAM6/8/MV.MA/7/AIZAO781/6/LOV11/SON64/4/PJ/GB55//093
18	0	R	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/6/YMH/HYS//HYS/TUR3055/3/DGA/4/VPM/MOS/5/TRAP#1/BOW
19	0	R	DORADE/ALTAY2000/5/VEE/TSI//GRK/3/NS55.03/4/SUZEN
20	0	R	**ERAYBEY**
21	0	R	BDME 09/1K
22	0	R	BDME 11/1K
23	0	R	BDME 11/2K
24	20	S	BDME 11/3K
25	40	MS	**MÜFİTBEY**
26	100	S	**LITTLE CLUB**

* Percentage of Flag Leaf Area Covered by Stripe Rust pustules

**S (Susceptible), MS (Moderately Susceptible), MR (Moderately Resistant), R (Resistant)

Development of resistant varieties against the disease is always an important objective in wheat breeding programs for crop improvement. These resistance genes, however, became ineffective due to the acquisition of virulence to that particular resistance gene (Sajid et al., 2014). As an alternative control method for controlling Stripe Rust, utilization of genetically resistance resources is inevitable way and in this regard, the importance of these studies emphasized one more time in this study.

Conclusion

In brief, the breeding for screening of resistant variety would be considered as long-term studies since some pathogens like Stripe Rust could change form and generate new pathogenic race often. It is needed to take precautions including the registered varieties for screening studies of resistance to prevent the disease attack and losing the resistant varieties.

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DETERMINATION OF OPTIMUM O_2 OR CO_2 FOR APPLE CV. STARKRIMSON DELICIOUS UNDER CONTROLLED ATMOSPHERE STORAGE

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Abstract

In developed countries, instead of regular atmosphere (RA) storage, controlled atmosphere (CA) storage is used for storing apples. In Turkey, Isparta is the major apple production area. However, CA storage is not widespread. Some of the main reasons why CA storage is not widespread are as follows: the effects on fruit quality of CA storage are not sufficiently understood, there is still a lack of knowledge on storage composition and conditions, and technical staff. This study was conducted for the purpose of determining optimum gas compositions for Starkrimson Delicious variety, which is widely produced in the region, under CA storage. The apple fruits subject to the study were harvested at optimal harvest date and then stored at 0°C for 8 months under both RA and CA storage conditions (1% CO_2, 2% O_2; 2% CO_2, 2% O_2; 3% CO_2, 2% O_2), followed by a shelf life of 7 days at 20°C. During storage periods, fruit quality analyses (firmness, weight loss, TSS, TA, ethylene production, respiration rate, fruit color) and biochemical analyses (antioxidant activity and total phenols content) were performed. The analyses made demonstrated that, compared to RA storage, all CA storage conditions were in general more effective in preserving the quality of fruits. CA storage led to reduced weight loss, suppression of ethylene production and respiratory rate, and maintenance of firmness. Antioxidant activity and total phenols content increased under both CA and RA storage conditions during storage. The results obtained indicate that the most suitable CA storage composition in maintaining apple quality factors is 2% O_2 + 3% CO_2.

Keywords: *Apple, Starkrimson Delicious, fruit quality, controlled atmosphere*

Introduction

The world apple production is approximately 76 million tons. The group of Red Delicious corresponds to 19% of the said production (Özongun et al., 2014). According to the data of 2014, Turkey is ranked as the third in the world apple production, with a production amounting to 2.480.444 tons (TUİK, 2015). Isparta province meets 22% of Turkey's apple production with 615.000 tons. Nearly half of the apples produced in Turkey are Red Delicious (Erkut, 2009). Starkrimson Delicious is an apple variety included in the said group.

Isparta is a significant province of Turkey in terms of apple production and cool storage rooms. In the province, apple fruits are commonly stored at regular atmosphere storage rooms. In recent years, there has been an increase in controlled atmosphere storage capacity. In line with this increase, researches conducted on storage conditions for the apple varieties grown in the region have become crucial.

In recent years, CA storage rooms have been used in order to maintain quality for a longer time and reduce losses during the storage of apples (Bai et al., 2005). The CA technology makes it possible to store products at higher quality for a longer time (Beaudry, 2009). In CA storage, ambient air composition at cool storage rooms (21% O_2, 0.03% CO_2 and 79% N_2) is modified by reducing oxygen and increasing carbon dioxide. Thus, cold storage and shelf life is extended for many fruits and vegetables compared to regular storage (Karaçalı, 2009). The performance of fruits in CA storage systems varies by type or even variety (Brackmann and

Streif, 1993). The effects of O_2 and CO_2 levels in atmosphere composition on a product depend on product species and variety, maturity phase in harvest, and gas concentration at the storage room (Thompson, 1998). In addition, product's sensitivity to low O_2 and high CO_2 under the ecological conditions of the region where the product is grown is also effective (Lau, 1985; Ertan et al., 1992; Volz et al., 1998). For this reason, in controlled atmosphere storage, optimum gas composition needs to be determined for each product according to the ecological conditions under which it is grown. Depending on species and variety, CA storage below or above the optimum leads to specific low oxygen and high carbon dioxide harms. On the other hand, anaerobic respiration and fermentation cause deteriorations in aroma, changes in taste, and irregularities in ripening (Karaçalı, 2009).
This study was aimed at determining the gas composition suitable for CA storage in the apple variety "Starkrimson Delicious", which is widely produced in Isparta (Eğirdir) region.

Material and methods

In the research, a 25-year-old Starkrimson Delicious on seedling rootstock in the Directorate of Eğirdir Fruit Research Institute was used as the material. Having been harvested at optimal harvest time, fruits were pre-cooled at 4°C. Following pre-cooling, fruits were stored in CA and in 3 different atmosphere compositions and under RA conditions for 8 months (Table 1). In order to determine the effects of treatments on shelf life quality, some of the samples taken every 2 months were kept for 7 days under shelf life conditions. The fruit samples taken every other 2 months during storage were analyzed in terms of weight loss (%), skin color (b* and h°), fruit flesh firmness (N), total soluble solids (TSS) (%), pH, titratable acidity (TA)(%), respiration rate (mL.CO_2/kg.h), ethylene production (µl.C_2H_4/kg.h), total phenols content (GAE mg/100 ml) and antioxidant activity (µmol TE/ml).

Table 1. Storage type, atmosphere composition and storage conditions

Storage type	Atmosphere composition		Storage conditions	
	CO_2 (%)	O_2 (%)	Temperature (°C)	Relative humidity (%)
CA-1	1	2	0	90-95
CA-2	2	2	0	90-95
CA-3	3	2	0	90-95
RA	0.03	21	0	85-90
Shelf life	0.03	21	20	60-65

Completely randomized design was replicated 3 times; 20 fruits per replication. The data obtained from the trial were subjected to variance analysis, using the JMP 7 statistical package program. Every storage period, differences between storage conditions averages were grouped according to the LSD multiple comparison test ($P<0.05$).

Results and Discussion

During storage periods, different levels of weight loss occurred in fruits under different storage conditions (fig 1). In terms of weight loss, there was a statistical difference between storage type averages. The highest weight loss occurred in regular atmosphere (RA) storage, while the lowest weight loss took place in CA-2. As reported in previous studies, fruits lose more weight in RA storage than CA storage (Erkan et al., 2004; Çalhan et al., 2014). The reason is that the relative humidity rate is lower in RA storage. The amount of weight loss continued to increase during shelf life. Especially in the fruits taken from CA storage rooms, the weight loss was higher than the one observed during storage.

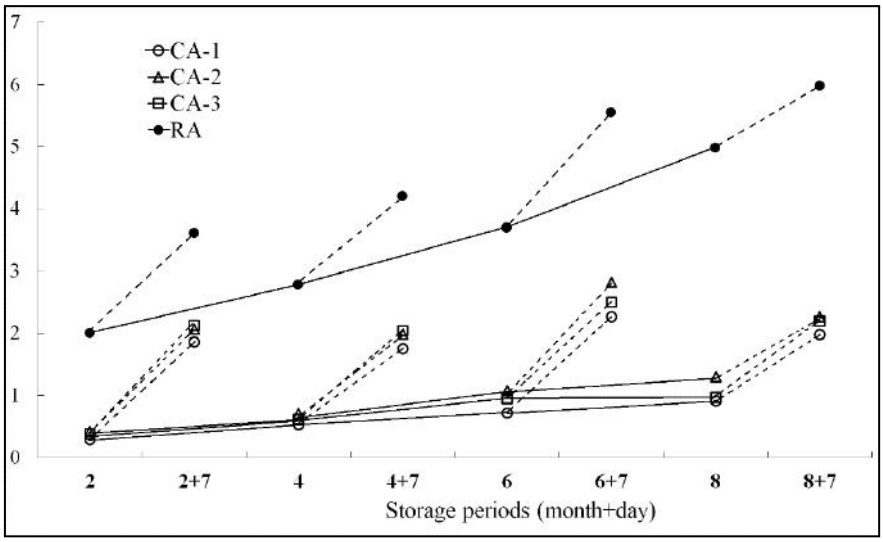

Fig. 1. Effects of different storage conditions and storage periods on weight loss of Starkrimson delicious apple fruits (dashed line: at the end of shelf life period)

Fruit flesh firmness is another quality criterion in apples significant for consumers. Important differences were found in terms of firmness both during storage period and between storage type (Table 2). Beginning from harvest, a decrease was encountered in firmness levels during storage period (Zanella, 2003; Çalhan et al., 2014). The decrease in question increased further during shelf life. Especially at CA storage, decrease in firmness considerably slowed down during cold storage (Erkan et al., 2004). Here, fruit flesh firmness was best preserved at CA-2 and CA-3 storage type. The level of CO_2 was higher in the said storage rooms compared to CA-1, affecting the decrease of firmness. At the end of cold storage, a difference of approximately 10 N occurred in firmness values between CA storage and RA storage. However, the difference decreased during shelf life.

Having been 11.2% during harvest, the amount of TSS in fruits first increased at all storage rooms and then slightly decreased towards the end of storage (Table 2). In RA storage, the decrease in the amount of TSS in fruits was slightly higher than other storages.

Taking part in the creation of taste and aroma in fruits, acidity is another quality criterion. If acidity is maintained specifically during storage, it means the fruit has been stored better. In our study, acidity in fruits continuously decreased compared to the acidity during harvest. The decrease in question was more rapid in RA storage than CA storage (Zanella, 2003; Fan et al., 1997). The best results in maintaining acidity in fruits were obtained under CA-2 conditions.

Starkrimson Delicious has a completely red skin. During storage, no considerable change was observed in fruit skin color. A difference was seen between storage types in the statistical analysis performed in terms of hº color value. The highest hº values were obtained from RA and RA + shelf life fruits. In general, CA storage conditions were more effective in preserving hº color values in fruits.

Table 2. Effects of different storage conditions and storage periods on firmness (N), SSC (%), acidity (g/100ml) skin color (h^o) of Starkrimson delicious apple fruits.

Storage conditions	Periods (month+day)	Firmness (N)	SSC (%)	Acidity (g/100ml)	Skin color (h^o)
	Harvest	75,2 a	11,2 c	0,26 a	32,3 c
CA-1	**8**	63,4 c	13,1 b	0,24 b	31,2 cd
	8+7	52,2 de	14,4 a	0,23 bc	25,6 e
CA-2	**8**	65,4 bc	14,4 a	0,26 a	31,4 cd
	8+7	51,6 e	14,2 a	0,26 a	28,5 de
CA-3	**8**	66,3 b	14,7 a	0,23 bc	32,9 bc
	8+7	52,6 de	14,5 a	0,22 c	27,0 e
RA	**8**	54,4 d	13,1 b	0,14 e	35,8 ab
	8+7	50,8 e	13,9 ab	0,17 d	36,6 a
Significant		***	*	***	***
LSD		2,47	0,91	0,018	3,10

* Level of significance: *P≤0.05; **P≤0.01; ***P≤0.001.

The ethylene production of fruits was 0,31 µl/kg.h during harvest. However, during storage and especially RA storage, it increased very rapidly and reached ~80 µl/kg.h. On the other hand, in CA storage, ethylene production was suppressed and increased up to 15-25 µl/kg.h during cold storage. Previous studies also reported that CA storage suppressed ethylene production in apples (Wünsche and Heyn, 2015; Fan et al., 1997). It was seen that ethylene production decreased as the level of CO_2 increased between CA storage types. The lowest levels of ethylene production were measured under CA-3 conditions. CO_2 was seen to suppress ethylene production in fruits. During shelf life, the level of ethylene production increased in the fruits taken from CA storage but remained the same in the fruits taken from RA storage. However, the level of ethylene production of the fruits at CA storage rooms was found to be lower. During shelf life, the fruits stored at CA storage rooms were statistically in the same group.

Respiration rate is one of the most important factors determining the life of fruits after harvest. Increased respiration rate means a shorter post-harvest life. For this reason, the more the respiration rate of a fruit is suppressed under different storage conditions, the longer the fruit is stored. In our study, fruit respiration rates continuously increased in RA storage but first decreased and then returned to the levels during harvest in CA storage. In CA storage, fruit respiration decelerates as O_2 decreases and CO_2 increases (Fan et al., 1997). In our study, no statistical difference was found between CA storage rooms. However, the lowest respiration rate was obtained from CA-3 conditions. During shelf life, fruit respiration rates slightly increased compared to cold storage. The said increase results from keeping fruits at 20ºC in shelf life.

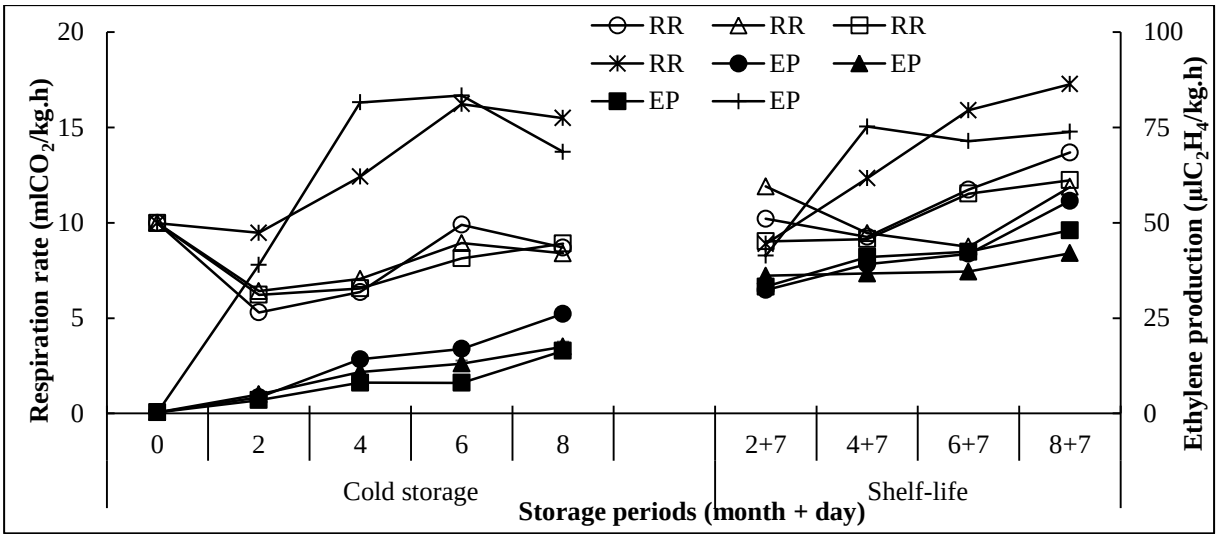

Fig. 2. Effects of different storage conditions and storage periods on respiration rate (RR) (ml CO_2/kg.h) and ethylene production (EP)(µlC_2H_4/kg.h) of Starkrimson delicious apple fruits.

During harvest, fruits' AA and TP contents were 6,17 µmol TE/g FW and 90,01 mg GAE/100 g FW, respectively. But, they increased during storage (Fawbush et al., 1997; Kuzucu and Aydın, 2014). At the end of storage, the highest increase was obtained in RA storage. An increase was also observed at CA storage rooms but it was lower than that at RA storage rooms.

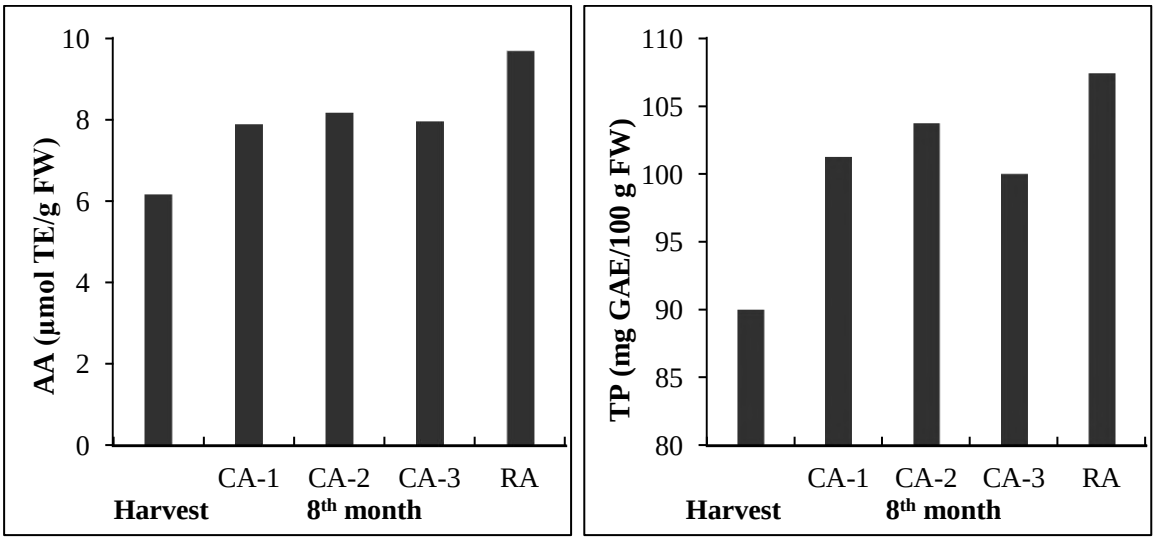

Fig. 3. Effects of different storage conditions on antioxidant activity (AA)(µmol TE/g FW) and total phenols (TP)(mg GAE/100 g FW) of Starkrimson delicious apple fruits at 8th month.

Conclusion

In Starkrimson Delicious, the level of weight loss did not exceed 5% for 8 months under both RA and CA storage conditions but the level of fruit flesh firmness highly decreased under RA conditions after the 6th month. For this reason, Starkrimson Delicious apple fruits cannot be stored for longer than 6 months under RA storage. For Starkrimson Delicious, during 8-month storage, CA-2 and CA-3 storage conditions were effective in preserving quality criteria (weight loss, ethylene production, respiration rate, fruit flesh firmness). However, the best storage condition was found to be CA-3 (2% O_2 + 3% CO_2).

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METAL, NON-METAL AND HEAVYMETAL CONTENTS OF CAPER PARTS

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Abstract

In current study, metal, non-metal and and heavy metal contents of different parts (buds, leaves, fruit and stems) of caper collected fromroadsideandinterior regionsof two different locations (Konya and Mersin) collected were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). K contents of samples changed between 10741 mg/Kg and 32529 mg/Kg. While the Ca contents of caper parts collected from roadside in Konya are found between 3344 mg/Kg (stem) and 17278 mg/Kg (leave), Ca contents of caper parts collected from interior regions was reported between 1762 mg/Kg (bud) and 10171 mg/Kg (leave). The same element was found between 4008 mg/Kg (stem) and 29104 mg/Kg (leave) on roadside in Silifke location. Cd contents of *C.ovata* and *C.spinosa* parts ranged from 0.06 mg/Kg (fruit) to 0.21 mg/Kg (stem) and 0.05 mg/Kg (fruit) to 0.54 mg/Kg (leave), respectively. Ni contents of *C.ovata* and *C.spinosa* parts changed between 0.37 mg/Kg (stem) to 1.53 mg/Kg (bud) and 0.19 mg/Kg (stem) to 4.91 mg/Kg (bud), respectively. In addition, while Zn contents of *C.ovata* change between 3.1 mg/Kg (stem) and 41.8 mg/Kg (leave), Zn contents of *C.spinosa* ranged from 11.5 mg/Kg (stem) to 44.6 mg/Kg (fruit).While the Cu contents of samples are found between 2.2 mg/Kg and 13.6 mg/Kg, Fe contents ranged from 32.9 mg/Kg to 117.7 mg/Kg.

Key words: caper, leave, fruit, heavy metal, road side, ICP-AES

Introduction

Caper is a plant with medicinal and aromatic properties. It is a long-lasting shruby plant that belongs to the Capparaceae family; capers occurs in various types (more than 350) and grows naturally in all the continents in many different regions of the world (Rodrigo et al., 1992; Özcan, 1996; Özcan, 2005). It is a tropical/subtropical plant (Rodrigo et al., 1992; Özcan and Akgül, 1998). The caper plant, which is called bubu, gebre, gabar, gevil, kapari, keper, kebere, turþuotu and Þebellah in different parts of Turkey, is an economically valuable plant. In various regions of the world different organs of caper species have been profitable for several purposes since ancient times. Young shoots, flower buds, and fruit are used for human nutrition. Capers have very important roles in the food industry; the flower buds are stored in brine and have become a costly product during recent years (Alvarruiz et al., 1990; Özcan, 1996). The composition of capers and especially *Capparis spinosa* have been widely evaluated showing that palmitic, linoleic acid, linolenic acid and sterols are the major components. In addition, rutin and kaemferol-3-rutinoside are the most abundant flavonol glucosides (Özcan, 1996; Inocencio et al., 2000; Giuffrida et al., 2002; Tlili et al., 2010). In the studies carried out about the nutritional value of caper. It was determined that the flower buds of caper (*C. sipinosa*) contained water, raw cellulose, vitamins, oil, fatty acids, protein, amino acids, and mineral substance (Costro Ramos and Nosti Vega, 1987). Caper organs such as buds, fruit and young shoots were found to be important sources of nutrients and essential elements. In addition, it is apparent that caper parts are good sources

of the macro and micro minerals, and consumed as a food ingredient to provide the human nutrition. The aim of current study is to determine heavy metal contents of caper parts contaminated exzost fumes in roadside and interior regionsof different areasfromtwo different locations.

Material and methods

Material

The parts (stem, leave, bud and fruit) of *Capparis ovata* Desf. Heywood and *Capparis spinosa* var. *spinosa* were harvested from roadside and interior regions of different areas in Konya (Selçuklu) and Mersin (Silifke) in Turkey, respectively. They were kept in cooled bags for transport to the laboratory. The samples were cleaned, and were dried under clear air conditions. Each sample was ground in a mill, and kept in colour vial till analyses.

Determination of mineral contents

Caper samples were dried at 70 °C in a drying cabinet with air-circulation until they reached constant weight. Later, about 0.5 g dried and ground sample was digested by using 5 ml of 65 % HNO_3 (Merck, Darmstadt, Germany) and 2 ml of 35 % H_2O_2 (Merck) in a closed microwave system (Cem-MARS Xpress, Matthews NC, USA) at 200 °C. The volumes of the digested samples were completed to 20 ml with ultra-deionized water, and mineral contents were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (Varian-Vista, Australia). Measurements of mineral concentrations were checked using the certified values of related minerals in the reference samples received from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) (Skujins, 1998).

Working conditions of ICP-AES:

In this study, ICP-AES (Varian-Vista) was used. Its RF Power changes between 0.7 and 1.5 kw (1.2-1.3 kw for Axial). In addition, plasma gas flow rate (Ar) ranged from 10.5 to 15 L/min. (radial) 15 “ (Axial). Auxilary gas flow rate (Ar) is 1.5 “. Viewing height is between 5 and 12 mm. Copy and reading time change between 1 and 5 s (max.60 s).

Statistical analyses

Results of the research were analysed for statistical significance by analysis of variance (Püskülcü and Ýkiz, 1989).

Results and Discussion

Mineraland heavy metal contents of different parts (buds, leaves, fruit and stems) of caper collected from road side and interior regions of different areasfromtwo different locations collected is given inTable 1. In general, it was observed that differences in element contents depend on plant partsand thelocations. Element contents of leaves were found high when compared with other parts. B, Cd, Cr, Mn, Mo, and Ni contents of caper leaves collected from the roadsideat the Konya locationwere found higherthancollected from the interior (off the road) areas. Cr, Fe, Mn and Ni contents of caper buds collected from the roadsideat the Konya locationwere determined higher than collected from the interior(off the road) areas of the same location. InSilifkelocation, Cr, Fe, Mn and Ni contents of caper buds collected from the roadsidewere determined higherthan those collected from the interior(off the road) areas of the same location. In addition, B, Cd, Cu, Fe, Mo and Zn contents of caper fruits collected in the interior(off the road) areas of the same location were established higherthan those collected from the roadside areas of the same location. OnlyMn andNicontents of caper fruits growing inroadsidewere found higherthan others. It is intersting that B, Cd, Cr, Cu, Fe, Mo, Ni and Zn contents of fruit stems collected from both inside fields were found higher compared with fruit stems on the road side. As a major elements, Ca, K, Mg, P and S were established in all samples. Of elements, K was found of the highest levels, and between 10741

mg/Kg and 32529 mg/Kg. Generally, K contents of stems were determined higher than those of other caper parts. It was followed by S, P, Ca, and Mg. While the Ca contents of caper parts collected from roadside in onya are found between 3344 mg/Kg (stem) and 17278 mg/Kg (leave), it was found between 1762 mg/Kg (bud) and 10171 mg/Kg (leave) in inside fields. The same element was found between 4008 mg/Kg (stem) and 29104 mg/Kg (leave) on roadside in Silifke location. In addition, this element in inside field at the same location was determined between 4231 mg/Kg (fruit) and 13789 mg/Kg (leave). Generally, while K contents of all samples change between 10741 mg/Kg (stem in Konya roadside) and 32529 m/Kg (stem in Silifke roadside), P ranged from 153 mg/Kg (stem in Konya road side) and 5405 mg/Kg (fruit in Silifke inside field). Ni contents of *C.ovata* and *C.spinosa* parts changed between 0.37 mg/Kg (stem) to 1.53 mg/Kg (bud) and 0.19 mg/Kg (stem) to 4.91 mg/Kg (bud), respectively. In addition, while Zn contents of *C.ovata* change between 3.1 mg/Kg (stem) and 41.8 mg/Kg (leave), Zn contents of *C.spinosa* ranged from 11.5 mg/Kg (stem) to 44.6 mg/Kg (fruit).While the Cu contents of samples vary between 2.2 mg/Kg and 13.6 mg/Kg, Fe contents ranged from 32.9 mg/Kg to 117.7 mg/Kg. The Ca, Fe, K, Mg, P and Zn levels are adequate. In previous study (Hamurcu et al., 2010), mineral and heavy metal levels of some fruits grown at the roadsides were established , and the results showed that the average level of Cu changed between 0.27 mg/kg (Sample 11) and 0.05 mg/kg (Sample 15), Cr 0.32 mg/kg (Sample 14) and 0.18 mg/kg (Sample 13), Ni 0.68 mg/kg (Sample 12) and 0.26 mg/kg (Sample 15), Pb 2.86 mg/kg (Sample 12) and 1.54 mg/kg (Sample 4) and Se 12.96 mg/kg (Sample 14) and 5.42 mg/kg (Sample 7).The levels of Cu, Cd and Cr in samples do not appear to reach pollution levels. Delibacak et al. (2002) researched the amounts of trace elements (Fe, Mn, Zn, Cu and B) and heavy metals (Co, Ni, Cr, Pb and Cd) of plants irrigated with water of Gediz river polluted by industrial and domestic wastes. In the vegetables from areas exposed to this pollution, copper was found in amounts from 0.10 to 3.26 mg/kg and zinc from 0.88 to 27.90 mg/kg. Copper in vegetables, fruit and cereals from areas differing in the degree of industrial pollution and from greenhouses copper amounted to 0.14-1.20 mg/kg and zinc from 1.0 to 10.60 mg/kg (Szymczak et al., 1993). Calcium is the major component of bone and assists in teeth development (Brody, 1994). The essential role of selenium for human health has been well established in recent years (Foster et al., 1998). Selenium has an active role as a modulator in inflammatory and immune responses (Neve,1991). The differences in chemical properties, mineral contents of caper parts belong to both plants were probably due to environmental conditions in conjunction with the analytical methods used.

Conclusion

As a results, relatively highoccurrenceof heavy metalsin samples collectedfrom the roadside,it wasprobably caused byvehicle emissions. Relatively highoccurrenceof heavy metalsin samples collectedfrom the roadside in Silifke location could be due the exhaust fumes of vehicles, due to close to inland regions, thetrafficto be more density in this location. In addition,the prevailing windscould have causedcontamination by dragging onto theplantof theexhaust fumes. Higher occurence of these values in the leavesmay be duemoreto be contaminated compared totheother organs ofthecaper and larger surface of leaves. Theexcessof the other elementsmay have probably resulted from nutrients,varieties and genetic structure. The main sources of heavy metals in plants are their growth media, nutrients, agro inputs and soil. Other sources may include pesticides and fertilizers. It is concluded that heavy metal contents of soil and emissions from vehicles may be the major source of heavy metal contamination. The levels of Cu, Cd and Cr in most of the analysed samples do not appear to reach pollution levels.

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Table 1. Mineral contents of caper parts (mg/Kg)*

	Samples	Ca	K	Mg	P
Konya (Selçuklu) Road side	Bud	4389 ± 155**	24099 ± 208	2966 ± 143	4340 ± 183
	Leave	17278 ± 279	24472 ± 775	3943 ± 31	2775 ± 172
	Fruit	3626 ± 75	21981 ± 856	2887 ± 165	3842 ± 101
	Stem	3344 ± 108	10741 ± 140	749 ± 41	153 ± 15
Konya (Selçuklu) Interior region side	Bud	1762 ± 67	23475 ± 1558	2422 ± 104	4281 ± 208
	Leave	10171 ± 249	23267 ± 461	3754 ± 53	4099 ± 23
	Fruit	1799 ± 82	21482 ± 626	2492 ± 110	4291 ± 202
	Stem	4500 ± 180	26694 ± 1778	3178 ± 266	2101 ± 30
Mersin (Silifke) Road side	Bud	6525 ± 369	24181 ± 754	2893 ± 258	4228 ± 184
	Leave	29104 ± 320	22529 ± 736	3976 ± 93	1220 ± 112
	Fruit	4040 ± 168	22600 ± 58	2328 ± 66	2528 ± 258
	Stem	4008 ± 155	32529 ± 2188	2702 ± 172	1334 ± 75
Mersin (Silifke) Interior regions side	Bud	6826 ± 337	28120 ± 439	3046 ± 47	5224 ± 333
	Leave	13789 ± 358	26767 ± 158	3551 ± 70	4099 ± 151
	Fruit	4231 ± 61	25250 ± 1359	3037 ± 77	5405 ± 532
	Stem	5232 ± 424	30544 ± 1522	3306 ± 229	3208 ± 126

*dry weight
**mean±standard deviation

Table 2. Mineral contents of metal, non-metal and heavy heavy metal (mg/Kg)*

	Samples	B	Cd	Cr	Cu	Fe	Mn	Mo	Ni	S	Zn
Konya (Selçuklu) Road side	Bud	18.4 ± 0.9**	0.08 ± 0.01	0.89 ± 0.07	7.5 ± 0.6	64.4 ± 6.6	20.0 ± 1.7	0.27 ± 0.02	1.53 ± 0.07	16465 ± 533	35.3 ± 1.0
	Leave	51.8 ± 3.2	0.19 ± 0.01	0.90 ± 0.07	6.0 ± 0.1	76.8 ± 1.4	53.5 ± 3.6	0.83 ± 0.03	1.21 ± 0.11	27518 ± 736	35.4 ± 3.4
	Fruit	18.5 ± 0.7	0.06 ± 0.01	0.48 ± 0.06	8.1 ± 0.3	52.2 ± 1.8	18.0 ± 1.5	0.43 ± 0.07	0.84 ± 0.06	14130 ± 1044	39.1 ± 2.9
	Stem	10.9 ± 0.9	0.12 ± 0.02	0.24 ± 0.02	2.2 ± 0.3	32.9 ± 3.4	10.2 ± 1.0	0.13 ± 0.03	0.38 ± 0.02	3588 ± 315	3.1 ± 0.3
Interior region	Bud	21.5 ± 0.8	0.09 ± 0.01	0.53 ± 0.09	11.5 ± 0.7	60.7 ± 4.1	14.7 ± 1.5	0.35 ± 0.02	1.21 ± 0.19	13403 ± 1462	35.8 ± 1.6
	Leave	42.7 ± 0.8	0.16 ± 0.00	0.37 ± 0.02	13.6 ± 0.4	138.2 ± 4.7	26.9 ± 0.6	0.58 ± 0.02	1.12 ± 0.09	20253 ± 108	41.8 ± 0.3
	Fruit	16.3 ± 0.7	0.06 ± 0.01	0.16 ± 0.01	9.4 ± 0.1	45.8 ± 3.6	12.5 ± 1.0	0.43 ± 0.02	0.41 ± 0.03	13875 ± 1230	34.8 ± 2.0
	Stem	17.7 ± 1.4	0.21 ± 0.03	0.27 ± 0.03	7.8 ± 0.8	49.1 ± 4.8	15.5 ± 1.0	0.30 ± 0.02	0.37 ± 0.03	14554 ± 1647	22.3 ± 1.4
Mersin (Silifke) Road side	Bud	18.5 ± 0.8	0.08 ± 0.01	0.22 ± 0.05	8.1 ± 0.1	60.7 ± 4.6	34.2 ± 1.9	1.15 ± 0.06	4.91 ± 0.54	17430 ± 1135	41.8 ± 1.4
	Leave	57.0 ± 4.6	0.15 ± 0.03	0.32 ± 0.05	5.0 ± 0.1	117.7 ± 3.6	102.9 ± 9.7	1.15 ± 0.58	3.08 ± 0.13	17726 ± 485	21.8 ± 0.3
	Fruit	16.5 ± 0.5	0.05 ± 0.01	0.33 ± 0.44	6.6 ± 0.1	50.5 ± 1.8	15.9 ± 1.5	0.41 ± 0.04	2.92 ± 0.27	13242 ± 677	22.8 ± 0.9
	Stem	17.5 ± 1.1	0.15 ± 0.00	0.20 ± 0.01	3.9 ± 0.4	33.2 ± 2.3	15.0 ± 1.6	0.35 ± 0.01	0.19 ± 0.03	18133 ± 1430	11.5 ± 1.1
Interior region	Bud	19.2 ± 0.6	0.22 ± 0.02	0.28 ± 0.04	4.3 ± 0.7	56.3 ± 1.6	22.5 ± 0.8	1.02 ± 0.04	0.67 ± 0.04	20948 ± 441	41.0 ± 0.8
	Leave	23.1 ± 0.5	0.54 ± 0.00	0.41 ± 0.03	6.3 ± 0.1	96.9 ± 6.0	31.9 ± 1.9	1.67 ± 0.04	0.66 ± 0.13	21115 ± 399	44.3 ± 1.9
	Fruit	20.7 ± 0.7	0.29 ± 0.02	0.28 ± 0.03	8.2 ± 0.6	55.1 ± 1.0	15.6 ± 2.1	0.90 ± 0.06	0.57 ± 0.08	14093 ± 1346	44.6 ± 0.9
	Stem	20.6 ± 0.5	0.51 ± 0.01	0.26 ± 0.02	5.1 ± 0.3	54.2 ± 3.9	11.4 ± 0.9	0.73 ± 0.03	0.33 ± 0.04	13434 ± 1325	18.7 ± 1.5

*dry weight

**mean±standard deviation

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DETERMINATION OF STORAGE AND SHELF LIFE QUALITY OF JEROMINE APPLE VARIETY GROWN IN THE ISPARTA

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Abstract

Jeromine is apple variety which is new breeding by mutation from Early Red One®. Isparta region is one of Turkey's most important apple-growing regions. Due to new varieties that are breeding introduce to this region, determining of storage performance of these varieties is necessary. With the aim of studying the influence of storage and shelf life quality of Jeromine apple variety, fruit stored at 0 °C and 85-90% relative humidity for 6 months. During cold storage, apple samples were removed at 4 and 6 months, and the half of them were analyzed, the other half were held at 20 °C and 60-65% relative humidity for 7 days to shelf life. In addition, immediately after harvest the half of fruit were treated with 625 ppb 1-methylcyclopropene (1-MCP) at 10 °C for 24 hours. Fruit weight loss, firmness, skin color, soluble solids content (SSC), titratable acidity (TA), ethylene production, respiration rate, α-farnesene and conjugated trienol (CT) concentrations and antioxidant activity and total phenols content were measured at intervals during storage and shelf life. Generally, firmness and TA decreased, and weight loss, ethylene production and respiration rates increased during storage of fruit, but 1-MCP markedly inhibited ethylene production and respiration rates. α-farnesene and CT concentrations of 1-MCP treated fruit were low. 1-MCP treated suppressed superficial scald in apple during both cold storage and shelf life. Especially, quality of untreated fruit was lower than 1-MCP treated. However, Jeromine apple variety stored quality manner for 6 months with both treatment.

Keywords: *Apple, Jeromine, fruit quality, storage, 1-MCP*

Introduction

Turkey, which ranks 3rd in the world, is one of the major apple producing countries, with 3.128 thousand tons apple production. Commercial apple production was continued traditional varieties such as Starking Delicious, Golden Delicious, Granny Smith, etc. in Turkey. However, in recent years, new breeding apple varieties started to introduce to Turkey. Commercial orchards were started to plant with this new apple varieties. Jeromine apple variety is one of them.

Origin of Jeromine is French mutation of Early Red One® Erovan. General characteristics: standard form of medium-low vigour extremely productive, fruit uniformly dark red, high quality. Ripens at the same time as Early Red One® Erovan*, about 5 days before Red Delicious spur types. Fruit is conic, symmetric, slightly ribbed; large size, higher quality than other Red Delicious clones (Anonymous, 2015).

1-methylcyclopropene (1-MCP), an inhibitor of ethylene biosynthesis, binds irreversibly to ethylene receptors in plants (Blankenship and Dole, 2003). Positive effect on postharvest fruit quality of 1-MCP was studied by a lot of researchers (Sisler et al.,1999; Fan et al., 1999; Watkins, 2006). 1-MCP-treated apples are exhibited more firm, more acidity, less ethylene production and respiration rate, less de-greening than untreated during both storage and shelf life (Çalhan et al., 2014).

Objective of this research was to investigate the responses to storage duration, 1-MCP treatment, and shelf life on fruit quality of Jeromine apples variety grown in Isparta.

Material and methods

Apples [*Malus sylvestris* var. *domestica* (Borkh.) cv. Jeromine] were harvested in a commercial orchard in Eğirdir, Isparta. Assessment of fruit maturity (starch index, firmness) was performed and then fruits were harvested on 22.9.2014. Fruit was exposed the day of harvest to 625 ppb 1-MCP at 10 ºC for 24 h in plastic bag. SmartFresh (AgroFresh, Inc.,) tablet was used to generate 1-MCP gas. After 24 h, the plastic bag was vented. Untreated and treated fruits were stored at 85-90 % relative humidity at 0 ºC for 6 months in regular atmosphere (RA). Stored fruits were taken from cold rooms at 0, 4 and 6 month, and then the fruits held for 7 days to simulate shelf-life (20 ºC, 60-65% RH) and analyzed.

Quality parameters

Weight loss was measured on 15 fruits during cold storage and shelf life. Results were expressed as % of weight loss relative to the initial weight.

Firmness was defined as the maximum load required to push the 11 mm probe into the fruit flesh (after skin removal) on two opposite sides of each fruit, to a depth of 10 mm (Güss FTA Type GS14, Strand, South Africa). The results were expressed in Newton (N).

Skin color was determined using a colorimeter CR-400 model (Minolta Co. Ltd., Japan) and expressed as L*, a*, b*, C* and hº.

The concentration of soluble solids (% SSC) and titratable acidity (TA) were performed with a refractometer (HI 96801 model Hanna, UK) and with a automated Titrator (T50, Mettler Toledo), respectively. NaOH 0.1 N was used to titrate 5 ml of juice + 45 ml pure water to pH 8.1. The results were calculated as malic acid equivalents in g/100 ml.

The ethylene production and respiration rate of fruit was measured with a gas chromatography (Agilent 6840) with equipment column FID and TCD. Ethylene production and respiration rate expressed as μLC_2H_4/kg.h and $mLCO_2$/kg.h, respectively.

Severity of superficial scald was assessed visually according to Jung and Watkins (2008).

α-farnesene and conjugated trionels (CTols) analysis method of Isidoro and Almeida, (2006) was used with modifications. Absorbances of appropriate dilutions of the hexane extracts were measured at 232 nm (α-farnesene) and at 258, 269, 281 and 290 nm (CTols) using a UV/Vis spectrophotometer (Cary® 50 model, Varian). Concentrations of α-farnesene were calculated using a molar extinction coefficient of ε232= 27700 (Huelin and Coggiola, 1968) and CTols were calculated molar extinction coefficient of 25 000 (Anet, 1972) in $\mu g/cm^2$.

Total phenols (TP) were determined according to the Folin–Ciocalteu method (Waterhouse, 2002). Absorbance was measured at 725 nm in a UV-Vis spectrophotometer (Cary® 50 model, Varian). TP were expressed as mg gallic acid equivalents (GAE)/100 g FW. Antioxidant activity (AA) of apple phenolic extracts was measured using method of Benzie and Strain (1996) with some modification. Absorbance was measured at 515 nm. AA was calculated as μmol Trolox equivalents (TE)/g FW. All samples were analysed in triplicates.

Data were subjected to analysis of variance (ANOVA, JMP7), means were separated by mean of LSD test ($P<0.05, 0.01, 0.001$).

Results and Discussion

During both cold storage and shelf life, weight losses were increased, as expected (Çalhan, et al, 2014). Weight loss of 1-MCP-treated fruit was slightly lower (Table 1). It has been reported that while in some studies 1-MCP affected on weight loss (Özkaya and Dündar, 2009), in some studies 1-MCP did not affect (Porat et al., 1999).

1-MCP-treated fruit were consistently firmer than untreated fruit during both cold storage and shelf life. Like other similar findings, this finding shows that 1-MCP effectively delayed apple firmness loss (Fan et al., 1999; Dell et al., 2002). Firmness of 1-MCP-treated apple higher than control fruit (~10 N) at the end of storage.
Firstly, SSC was increased from harvest to during storage periods. But, a tendency was observed to decrease in SSC towards at the end of storage. 1-MCP-treated apple had higher SSC than control fruit (Fan et al., 1999). TA declined throughout storage periods, but this was particularly noticeable in control treatment. A lot of studies were reported that loss of TA in 1-MCP-treated apple reduced (Fan et al., 1999; Wünsche and Heyn, 2015).

Table 1. Effects of treatments and storage periods on weight loss (%), firmness (N), SSC (%), acidity (g/100ml) of Jeromine apple fruits.

Treatments	**Periods**	**Weight loss (%)**		**Firmness (N)**		**SSC (%)**		**Acidity (g/100ml)**	
	Harvest			75,1 a		10,8 d		0,32 a	
Control	6	4,27	4,85	61,3 c	65,0	11,8 c	11,6 B	0,22 c	0,25 B
	6+7	5,43	A	58,7 d	B	12,3bc		0,21 c	
1-MCP	6	3,84	4,43	68,6 b	70,6	13,4 a	12,3 A	0,27 b	0,29 A
	6+7	5,02	B	68,3 b	A	12,6 b		0,28 b	
Significant		NS	*	***	***	**	**	***	***
LSD			0,35	2,44	1,56	0,41	0,71	0,02	0,01

* Level of significance: *P≤0.05; **P≤0.01; ***P≤0.001. NS: not significantly.

Respiration rate and ethylene production of control fruit increased during storage periods, while they were reduced substantially by 1-MCP treatment (Fig 1). Ethylene production was fifty fold at 6^{th} month and nine fold at $6+4^{th}$ month+day greater in control fruit than in 1-MCP-treated fruit. This is in agreement with the finding that 1-MCP reduces ethylene production in fruit at both cold storage and shelf life (Fan et al., 1999; Çalhan et al., 2014). During storage periods, respiration rate of 1-MCP-treated lower than control fruit and respiration rate was around half that of control fruit.

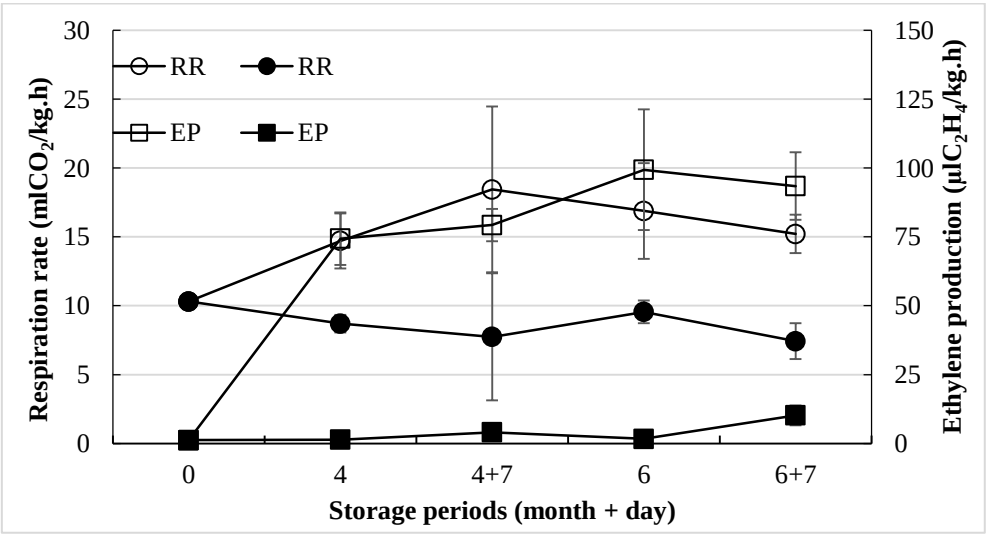

Fig. 1. Effects of treatments and storage periods on respiration rate (RR) ($mlCO_2/kg.h$) and ethylene production (EP) ($\mu lC_2H_4/kg.h$) of Jeromine apple fruits. Vertical bars represent standard deviation of the means.

Superficial scald severity was just significantly different (P<0.01) at 0.94 and 0 at the end of storage for control and 1-MCP-treated fruit, respectively (Fig 2). It was observed that 1-MCP inhibited superficial scald during storage periods (6 months + 7 days) (Zanella, 2003). The concentration of α-farnesene in peel tissue had continued to increase over storage in both treatments, but this increase was higher in control fruit than 1-MCP-treated (Fig. 2). α-farnesene of control apples increased dramatically during the initial of storage, and then declining (Jung and Watkins, 2008; Çalhan, et al, 2014). The level of CTols remained high in control fruit and was three fold greater than in 1-MCP-treated fruit (Watkins et al., 2000).

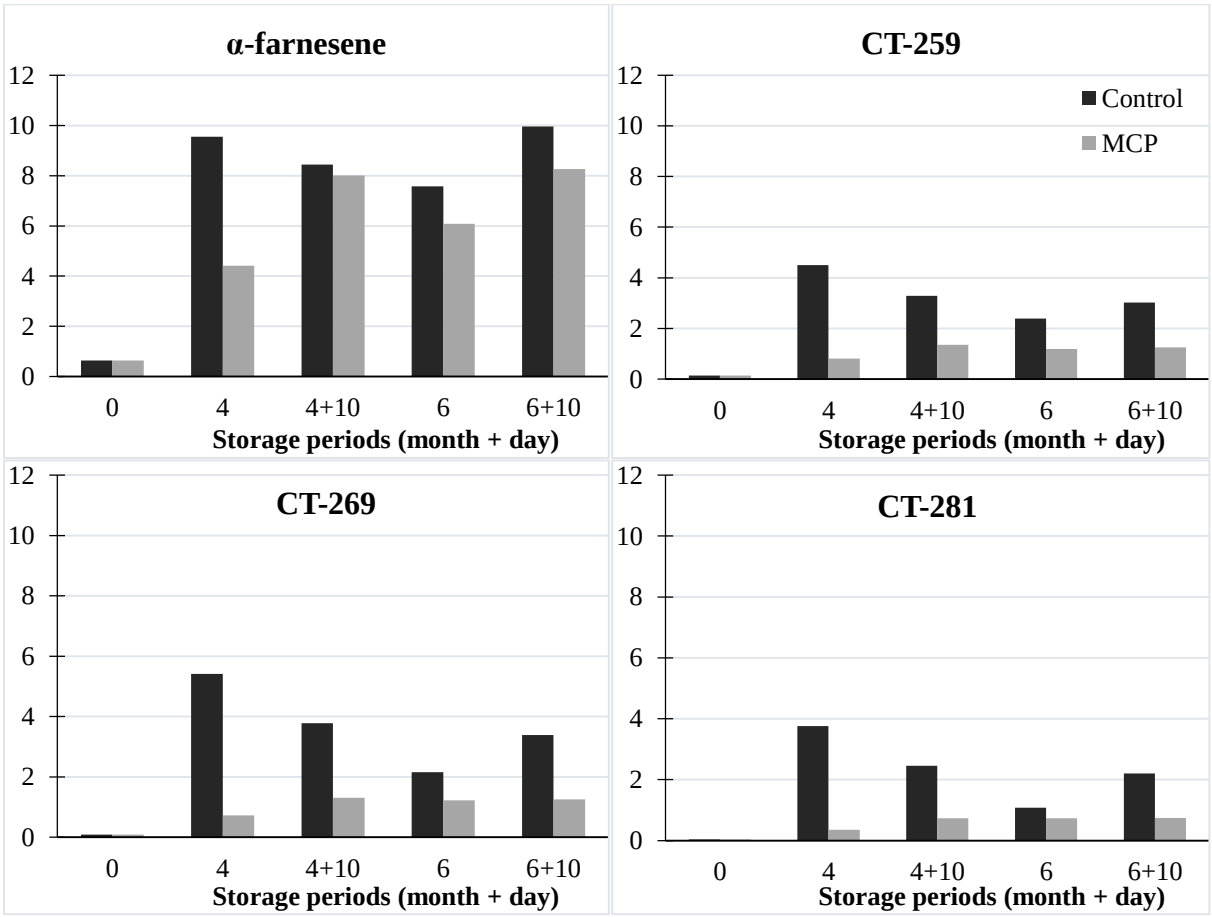

Fig. 2. Effects of treatments and storage periods on α-farnesene, CT-259, CT-269 and CT-281 (μg/cm^2) of Jeromine apple fruits.

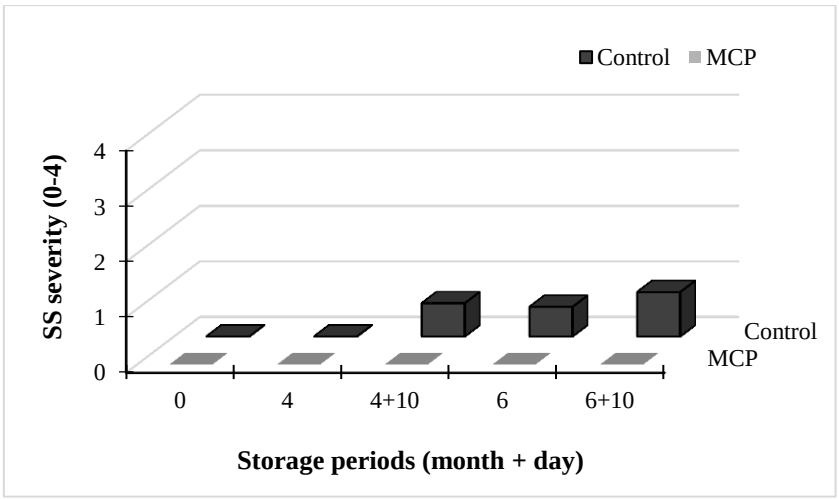

Fig. 3. Effects of treatments and storage periods on superficial scald severity of Jeromine apple fruits.

The AO and TP content of 1-MCP-treated fruits lower than control fruit during storage periods. The AO and TP content of both control and 1-MCP-treated fruit higher at cold storage than shelf life. AA and TP of Jeromine apple slightly decreased during storage periods (Fig. 4). Previous studies described different response of apple TP to cold storage and shelf life after removal from cold storage caused fluctuations in TP (Goulas et al., 2014).

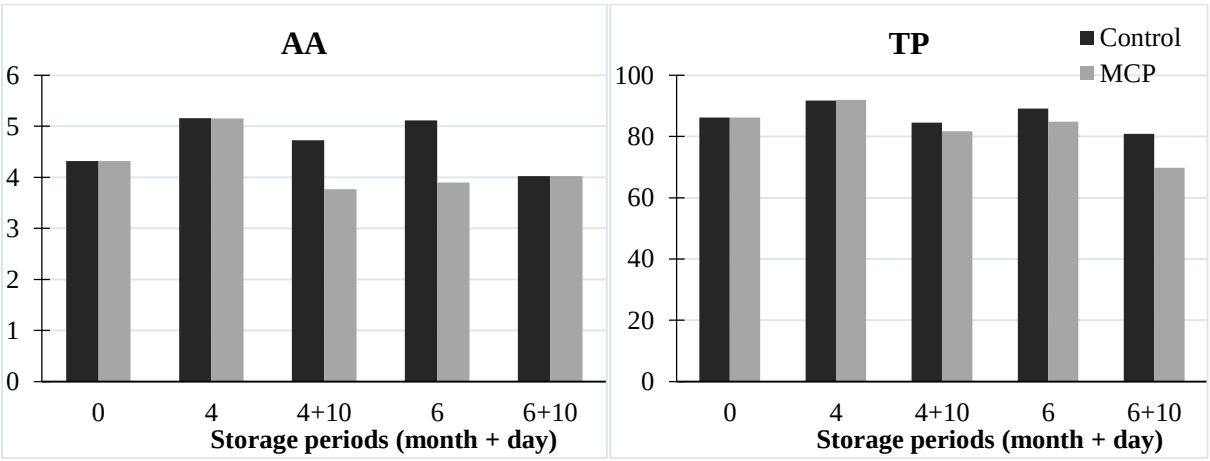

Fig. 4. Effects of treatments and storage periods on antioxidant activity (AA)(µmol TE/g FW) and total phenols (TP)(mg GAE/100 g FW) of Jeromine apple fruits.

Skin color of Jeromine apple did not clearly determine during RA storage and shelf life. With regard to all color values did not significantly difference between treatments (Data not shown). This was probably due to dark red skin color of Jeromine apple variety and skin color did not change throughout storage periods.

Conclusion

Jeromine apple stored in RA maintained a good firmness, skin color and weight loss. During RA storage, there was a clear difference in ethylene production and respiration rate between untreated and treated with 1-MCP. On the other hand, 1-MCP was more effective in preventing superficial scald. 1-MCP maintained firmness during both cold storage and shelf life. Our results show that Jeromine apple may be stored at 0 ºC for 6 month then at 20 ºC for 7 days shelf life. But, treated 1-MCP apple have higher fruit quality than untreated apple at the end of storage periods.

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Review paper
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COLOR QUALITY OF FRESH-CUT FRUITS AND VEGETABLES

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Abstract

Fruit and vegetables are attractive and eye-catching to a large degree because of the richness of pigments that they contain. Color is derived from the natural pigments in fruits and vegetables, many of which change as the plant proceeds through maturation and ripening. The primary pigments imparting color quality are the fat soluble chlorophylls (green) and carotenoids (yellow, orange, and red) and the water soluble anthocyanins (red, blue), flavonoids (yellow), and betalains (red). In addition, enzymatic and non-enzymatic browning reactions may result in the formation of water soluble brown, gray, and black colored pigments. Preservation of chlorophyll in vegetables, red to purple anthocyanins, yellow, orange and red carotenoids in both fruits and vegetables is of vital importance to maintain quality. The enzymes involved in browning reactions include polyphenol oxidase, which catalyzes the oxidation of polyphenolic compounds, and phenylalanine ammonia lyase, which catalyzes the synthesis of precursors to phenolic substrates. Color changes in fresh-cut fruits and vegetables may have different origins, for example decreased green pigmentation in fresh-cut lettuce may results from senescence, heat exposure or acidification; discoloration or browning of sliced mushrooms and sliced apples and pears is brought about through the action of polyphenol oxidases; white blush development in carrots is initially caused by desiccation, and then lignifications. In this study, color quality of fresh-cut fruit and vegetables, and the methods for preventing color changes was reviewed.

Keywords: *Fresh-cut, fruits, vegetables, color, browning, white blush, senescence.*

Introduction

Fresh-cut fruit and vegetable is defined that washed, trimmed, peeled, chopped or sliced, ready to use or cook, and packaged with suitable polymeric packaging material with covering tightly. These type foods are named such as fresh-cut, minimally processed, ready-to-use or ready-to-eat. (James and Ngarmsak, 2010; Barry-Ryan ve O'Beirne 1998).This group of food has emerged by variation of consumers demands in recent years.

In particular, because increase in labor intensity of working people, time limitations and increase in income level, the demand of ready-to-use food was increased. (Martin-Diana ve ark.2007). Also, high nutritional value of fresh fruit and vegetables has come to forefront each year. Especially, these products are rich in terms of beta-carotene, anthocyanins, lycopene, and antioxidants such as ascorbic acid. (Negi ve Roy 2000). The risk of developing chronic disease, also, decreased with consumption of these type food. (Li ve ark. 2000).

Fresh-cut method is used for widely in lettuce, cauliflower, carrots etc. since 1990's. But, the use of fresh-cut methods for fruits was began later because the difficulty of protecting quality. (Soliva-Fortuny ve Belloso, 2003). But, fresh cut technology is started to use also for fruits, recently. (Erbay, 2007 Oms-Oliu, 2010).

The sector of fresh-cut fruit and vegetables evolved over the last 20-30 years, and primarily in the United States it spread to the entire world. However, it was seen the fresh cut sector prominance both commercial and scientific in Canada, France and Italy. (Ergun 2006). Fresh-cut products markets has occured in USA, in the year of 2007, including 27 percent of

catering sector and 73 percent of consumer, and also $ 15.5 billion in total. (Cook 2008; James and Ngarmsak, 2010).

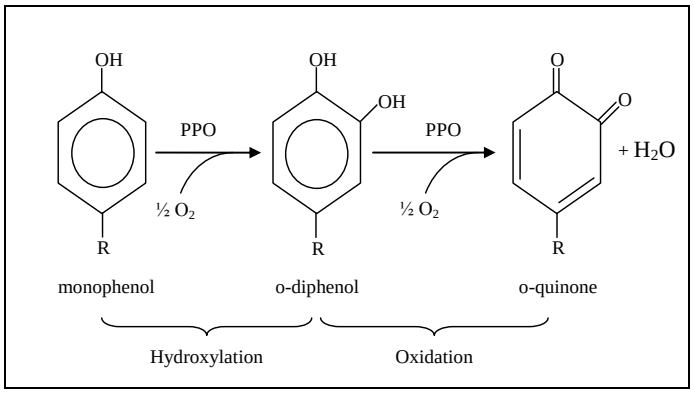
Appearance of fresh-cut fruit and vegetables is the most effective factor for the preference of consumer and purchase of produce. The visual quality of product is effected by many factors such as infections from microorganisms, wound tissue and discoloration. Even if these factors occurs different reasons or creates different results, eventually causes loss of product attractiveness. (Toivonen ve Brummell 2008). Visual quality changes in fresh-cut products are enzymatic browning, loss of chlorophyll, and microbial growth. In this study was compiled studies made to preserve the color.

The colors derived from natural pigments has much change during maturation process, in fruit and vegetables. Primary pigments that effecting color are fat soluble chlorophyll (green) and carotenoid (Yellow, orange and red), and water-soluble anthocyanins (red, blue), flavonoids (yellow) and betalaines (red). In addition, enzymatic and non-enzymatic browning reactions can cause grey or black color pigment formation. In the formation of enzymatic browning reactions, the enzymes of polyphenol oxidase and phanylalanine ammonia lyase that catalyse oxidation of phenolic substance are effective. Chlorophylls are sensitive against acidity and temperature, but are stable against alkaline conditions; whereas carotenoids susceptible to light and oxidation but relatively stabil against temperature. Carotenoids can be bleached by an enzyme called lipoxygenase which catalyzes the oxidation of lipid compounds. Flavonoids are sensitive to oxidation but relatively resistant to temperature; whereas anthocyanins are susceptible both pH and temperature, also betalains sensitive to temperature like anthocyanins. Moisture content of surface, wax formation and postharvest application has effected of brightness of fresh cut fruit and vegetables. (Barrett et al 2010)

Enzymatic Browning

The most important visual quality loss in all fresh-cut fruit and vegetables that especially have white flesh are browning occured in the cut surface. These browning reactions occured as result of reaction of polyphenol oxydase (PPO) and peroxydase (POD) enzymes with oxygene and phenols. (Toivonen and Brummell 2008).

PPO's are oxidoreductases containing copper. Currently, catechol oxidase, laccase and phloroglucinol were defined as three different PPO enzymes, and the most common PPO enzyme in plants kingdom is katechol oxydase. This enzyme has two activity including hydoxylation (krezolaz activity) and oxidation (catechol activity) (Fig. 1). Indeed; monophenols are transformed o-quinones through firstly hydroxylation and then oxidation. The o-quinones are formed trihydroxybenzenes (THB) through hydroxylation. Hydroquinones has formed by interacting of THB with o-quinones, and these compound turned firstly polimers red-brown colors and then melanins that brown color. (Cemeroğlu et al 2004).



Şekil 1. The mechanism of action of catechol oxidase (Toivonen and Brummell 2008).

Another group of enzymes that influence the browning are peroxidase (POD). Fruit and vegetables have peroxidase mostly containing iron. POD enzyme has different action. But activity of enzymes known as "hydroxylation effect" that diphenol formation from monophenols like PPO enzyme activity is not important. But the need of hydrogen donor for perform this operation, is the difference of POD's from PPO. POD caused discoloration, redution of taste and loss of nutritioral value according to subtrate used. (Cemeroğlu et al 2004). It was determined that POD caused oxidative breakdown of ascorbic acid (Zheng and Lu, 2011), bleaching of carotenoids (Song et al. 2010, Gandul-Rojas et. al. 2004) and betalains (Wasserman and Guilfoy, 1983), breakdown of chlorophyll (Martinez et al. 2001, Funamoto et al. 2003).

Control of Enzymatic Browning

Enzymatic browning could be controlled with both chemical or physical methods using alone or both of them. Physical methods are considered as reduction of temperature and/or oxygene, modified atmosphered packaging, edible coatings, gamma irradiation or high pressure. Some chemical compounds are used as chemical methods. The effects of these chemicals are to preventing of enzyme activity, to removing or inhibiting of activity of substrate (oxygene or phenolics). (Garcia and Barrett 2002).

Chemical Treatments

Initially, sulphites were used commonly. But they caused allergenic reactions and health risks, the using of them in fresh fruit and vegetables were banned by Food and Drug Administration (FDA) in USA at 1986 (Madinez and Whitaker, 1995; Taylor 1993). From this date it has been studied of many chemical compounds to preventing enzymatic browning.

Antibrowning Agents

Heat treatments are widely used to inhibition enzymatic browning in food processing industry. But, this method caused the losses of texture, taste and nutritional value of product. Therefore, some chemical compound was used to preventing of enzymatic browning which called as "antibrowning agents" (Garcia and Barrett, 2002). These agent are divided six group:

Acidulants:

For PPO enzyme activity, optimum pH range is considered 6.0-6.5; it is known that the enzyme activity was low below pH 4.5. Hence, product has lower pH or acidifier product is widely used for preventing enzymatic browning. (Garcia and Barrett 2002). Citric acid is the most widely used material for this purpose. Beside of citric acid; acetic acid, formic acid, fumaric acid, malonic acid, pruvic acid, oxaloacetic acid, succinic acid, tartaric acid, malic acid, oxalic acid, lactic acid, phosphoric acid, hydrochloric acid could be used (Son et al. 2001). As well as citric acid is used to preventing browning, it has slowing down effect on respiration. (Kato-Noguchi and Watada 1997).

Reducing Agents

This type of antibrowning agents were caused conversion of o-quinones to colorless diphenols through chemical reducing. During this reaction, reducing agent is oxidated irreversibly. This means that, they only provide a temporary protection due to they consume during reaction. If reducing agent is not added, the PPO activity begins again and browning occurs (Oms-Oliu et al. 2010). Thiol-containing compounds such as ascorbic acid and cysteine is included in this group. But, when these agent used together with calcium salts, the effectiveness is increased. (Arias et al. 2008, Gorny et al. 2002, Bieganska-marecik ve Czapski 2007, Rocha ve ark 1998)

Chelating Agents

Chelating agent prevents to PPO enzyme activity through forming a compounds with copper in PPO's active part. In this group, EDTA (ethylenediamine tetraacetic acid) (Arabacı 2015)

and a-phosphate-containing chelating agent is found. ((Garcia and Barrett 2002, Arabacı 2015).

Complexing agents

This group agent, have ability formation of complexing with reaction products and PPO substrates, or retention of them. Cyclodextrins (sugar molecules ring structure) and non-reducing cyclic oligosaccarides are used. (López-Nicolás et al. 2007).

Enzyme inhibitors

Sulphides are effective for activity of PPO enzyme, but using of them in fresh fruit and vegetables is forbidden. Na2So3, NaHSO3 and SO2 from them could be used. But, the residue level of SO2 should not exceed the permissible limits. It is also used as the PPO inhibitor of 4-Hexylresorcinol. (Piližota and Šubarić 1998).

Other antibrowning agents

Sodium chloride (NaCl), calcium treatments (Calcium chloride, calcium ascorbate, calcium lactate, calcium propionate) and substances such as honey are used for this purpose. (Son et al. 2001).

Physical Treatments

Low oxygene treatments

Living tissue is required oxygene. Fresh-cut products can not be held without oxygene environment because they maintain their viability. However, if oxygene is not present in reaction environment, enzymatic browning can be delayed. The cutting methods applied in fruit and vegetables are cutting of product under water, brine or syrup; so diffusion of oxygene to product was retarded. When contacting of the tissue with oxygen, the browning will be occured, in these methods (Garcia and Barrett, 2002). Modified atmosphere, controlled atmosphere conditions and edible coatings have been used for reducing oxygen. (Baldwin et al. 1995).

Heat Treatments

Low temperatures provides slowdown of metabolic activity in the product. It was effective including slowing of respiration rate, extending of shelf life and reducing activity of microorganism. However, low temperatures can delay activity of enzyme limited level (Siddiqui et al. 2011). Heat treatment is more effective for control of browning compared to low temperature storage. But, increases of temperatures, were caused sensory changes and tissue losses. Browning in pears cubes were prevented by heat treatments that applied in 95 C for 3 min, however, an acceptable reduction of texture has been occured. (Pittia et al 1999).

Gamma Irradiation Treatments

Gamma irradiation is a method for using quarantine purpose. It has effective reducing in microbial activity as well as inhibiting browning and losses of visual quality. It was found that gamma irradiation reduced browning of fresh-cut lettuce (Zhang et al. 2006) and the iceberg lettuce (Fan and Sokorai 2011).

High Pressure Treatments

High pressure applications are effective in inactivation of harmful enzymes. Variation of flavor quality and nutritional values of foods is very little changed or no changes obtained when this technology is used together with low temperature, (Garcia and Barrett 2002). Sometimes it can also be achieved that inactivation of enzyme when using antibrowning agent together with high pressure treatment (Guerrero-Beltrán et al. 2005). The activity of lipase, lypoxygenase, pectinmethylesterase, peroxidase and polyphenol oxidase enzymes were inactivated by high pressure processing (Siddiqi and Cash 2000), and losses of vitamins, pigments and flawor loss were remain minimum level(Weemaes ve ark., 1999).

UV Irradiation Treatments

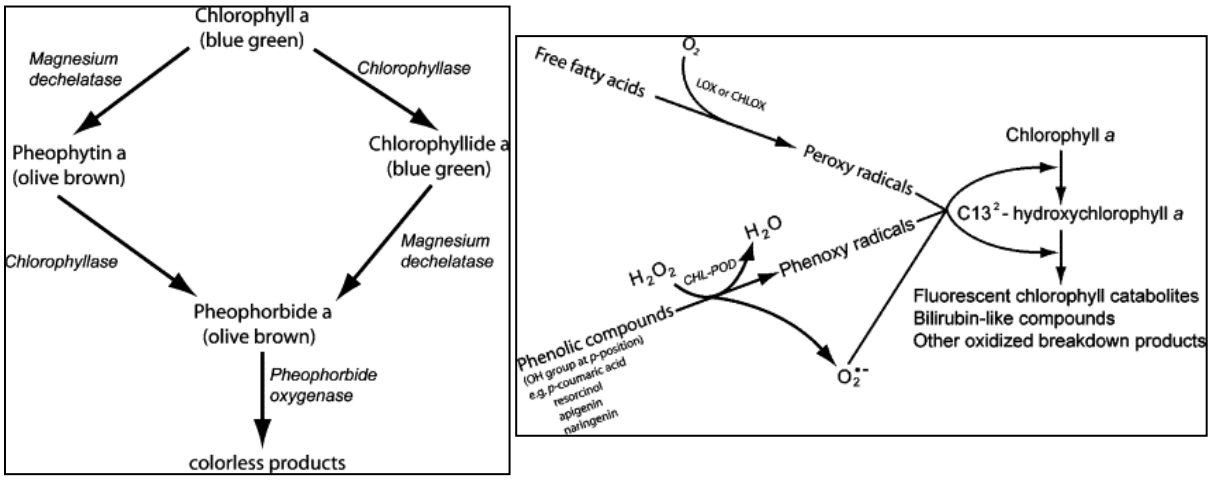
Different results were obtained by UV applications. Forexample, enzymatic browning of apple slices treated with UV-C for a few minutes was inhibited without quality loss (Manzocco et al 2009). But, Gomez et al. (2010) determined that browning of fresh-cut apple slices increased by UV-C treatments.

Whitening of Surface of Carrot (White Blush)

Whitening, is a special color variation that occured as result of drying of cut-surfaces during peeling and cutting, and also is named "White Blush". Although, drying is a physical process, "white blush" is occured in carrot surface in response to wounding of tissue. In fact, the process is associated with wound formation, this special process was starting by ethylene which was not part of lignification mechanism. Lignification is a serial process that was started by phenylalanine ammonia lyase, and involving numerous enzymatic transformations (Toivonen and Brummell 2008)."White blush" formation delayed by steam treatments, and also lignin formation retarded through producing soluble phenolic or isocoumarin (Howard et al. 1994). Also, it was determined that "white blush" formation was reduced by high relative humidiy of environment. (Cisneros-Zevallos et al 1995).

Loss of Chlorophyll or Yellowing

Yellowing is the emergence of colorless substances due to decreasing of the green pigment chlorophyll in fruit and vegetables during maturation and ripening. This type chlorophyll breakdown is named Type I fragmentation (Fig 2). While, colorless substance was formed on the one hand; the color pigment such as orange, purple or red on the other hand are synthesized during the maturation process. A major problem was encountered in many leafy vegetables including especially broccoli, brussel sprouts or spinach was yellowing. The methods widely used for the prevention yellowing during postharvest handling process are low temperature storage, heat treatments, exogenous cytokinin treatments, etyhlene inhibitors, modified atmosphere or controlled atmosphered storage (Zaicovski et al. 2008). Brown color substances was occured in the cut surfaces instead of colorless substances as results of chlorophyll breakdown. In this case, the degradation of chlorophyll is mediated by oxygene radicals. This type chloropyll breakdown is named as Type-II degradation (Fig. 2). In case of fresh-cut product, chlorophyll degradation appears as a form of Type II reactions (Toivonen and Brummell 2008).



Type I Type II

Fig. 2.Schematic representation of Type I and Type II chlorophyll breakdown pathways in green plant tissues (Toivonen and Brummell 2008).

Conclusion

In minimally processed fruit and vegetables; visual quality is the most important quality criteria for consumer. In this way color changes occured in fresh-cut fruit and vegetables are determined preference of consumer. There are three types of color changes are occuring in fresh-cut fruit and vegetables. Although, enzymatic browning has occured minimally processed fruit and vegetables, yellowing and whitening is the other color changes in mostly vegetables. For the preventing of color changes different methods were done, by researchers. In this study, color changes of minimally processed fruit and vegetables, and also preventing of these discoloration were investigated. For preventing of enzymatic browning or whitening of fresh-cut fruit and vegetables; either acidifiers or chelating agent were used varies fruit and vegetables. With the use of these chemicals for preventing color changes of fresh-cut fruit and vegetables; enzymatic browning, yellowing and whitening could be inhibited. But timely the effect of chemicals was decreased, and quality of fresh-cut fruit and vegetables were also decreased. Consequently studies about color changes of fresh-cut fruits and vegetables must be continued, for the consumer acceptance.

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Review paper
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THE METHODS TO PREVENT THE NEGATIVE EFFECTS OF ETHYLENE ON THE ETHYLENE-SENSITIVE CUT FLOWERS

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Abstract

Postharvest performance is of crucial importance to the value of cut flowers. The life span of flowers is generally determined by the time to abscission of petals that are still turgid, or by the time to petal wilting or withering. In many species, these phenomena are regulated by ethylene. Exposure to ethylene can reduce flower longevity by causing undesirable physiological disorders to vegetative and flowering organs. Ethylene-mediated premature floral senescence has been noted in several species, and many species have been shown to wilt in response to ethylene, including rose (Rosa hybrida), carnation, anthirhinum majus, orchids (Phalaenopsis), gerberas, lily flower etc.Premature senescence and abscission resulting from exposure to exogenous or endogenous ethylene can be controlled, both pre-treating flowers with inhibitors of ethylene biosynthesis and action.There are several inhibitors of ethylene biosynthesis, including aminooxyacetic acid (AOA) and aminoethovinylglycine (AVG). However, these chemicals are only effective against the effects of ethylene produced by the flower itself, and have no effect when flowers are exposed to exogenous ethylene, as can occur during transit and marketing. Therefore, the common treatments are of inhibitors of the response of flowers to ethylene. The negative effect of exogenous ethylene on cut flower quality was reduced with 2,5-norbornadiene (2,5-NBD), silver thiosulfate (STS) and 1-methycyclopropene.In this review, the effect of ethylene on cut flower vase life, and the methods to inhibiting the negative effect on cut flower longevity, was investigated.

Keywords: Ethylene, cut flower, silver thiosulphate, 1-methycyclopropene, vase life.

Introduction

The commercial value of cut flowers is determined by postharvest quality and long vase life expressed as color, brightness, turgority of flowers after harvest. Vase life of cut flower is defined as the time of abscission of petals that are still turgid, and by the time of petal wilting or withering (van Doorn, 2001). The reduction of vase life in most types of cut flowers associated with early petal senescence or wilting of petals, is caused with the effect of ethylene. Also, exposure to ethylene shorten cut flower vase life leading to unwanted physiological disorders in vegetative and floral organs.Ethylene one of the plant hormone that has growth inhibitor activity is synthesized in the plant during ripening and senescence process. Also, ethylene is released into the atmosphere that living plants as a result of ripening of fruits burning hydrocarbons such as propane and gasoline or via operation of construction machines. Ethylene, in the atmospheres that producing, transporting or storing area of flowers has detrimental effect on the flowers even low concentrations like 1 ppb (Reid 2000). In this study, cut flowers, that has special importance in Turkey and in the World trade, were investigated in terms of sensitivity of ethylene and the methods inhibiting ethylene effect.

Ethylene Sensitive Flower Species

In the studies conducted in order to determine the ethylene-sensitive flowers species (Woltering and Van Doorn, 1988), it was found to be there are 93 species of several families are ethylene sensitive. Petal abscission of these species was controlled by endogenous ethylene production. Flowers that belongs to families of Labiate (Lamiaceae), Ranunculaceae, and Rosaceae were showed ethylene-dependent petal abscission. Some families such as Orchidaceae showed petal wilting dependent of ethylene.

Ethylene-sensitive flowers are grouped into three categories. Senescence of the flowers in the first group is occured as a result of increasing ethylene production after pollination or during flower growth. The examples of flowers showing this way senescence can be given as carnation and petunia (Serek et al. 1995a). In the flower species like cyclamen in the second group, ethylene only showed increase during pollination and flowers have became sensitive against ethylene (Halevy et al. 1984). Flowers (eg. rose) in the third group, showed ethylene sensitivity during opening of flower bud while the production of ethylene was not show increase during senescence (Kumar et al. 2008).

When cut flower species exposed to ethylene, different response occurs. Petal wilting is occured as response to ethylene in the orchid (Palaenopsis) hibiscus (Çelikel and Reid 2002a) and carnation (*Dianthus caryophyllus*, Serek et al., 1995b). In the *Anthirhinum majus*, *Rosa hybrida* (Serek et al. 1995 a) and waxflower (*Chamelaucium uncinatum*, Macnish et el. 2000), ethylene encouraged petal or flower abscission. Cut flowers are affected at different rates from ethylene. Ethylene-sensitivity classes of cut flowers according to van Doorn (2001); (a) non-sensitive, (b) low-sensitive, (c) moderately sensitive, (d) highly sensitiveand (e) very highly sensitive as have been found (Table 1).

Table 1. The effect of ethylene on some cut flower species, and ethylene-sensitivity.

Family	Species	Ethylene effect	Sensitivity class
Amaryllidaceae	*Alstroemeria pelegrina*	*Perianth wilting, abscission*	Moderate/high
	Narcissus pseudo-narcissus	*Wilting, withering*	Moderate
Asphodelaceae	*Kniphofia* (hybrid)		Low sensitive
Cannaceae	*Canna* hybrid (3 variety)		Non-sensitive
Caryophyllaceae	Carnation	*Sleepiness of buds, non-opening, petal inrolling*	High sensitive
Iridaceae (Gladioleae)	*Gladiolus* hybrid	*Wilting, abscission*	Low/non sensitive
Ixieae	*Freesia* hybrid		Non-sensitive
Liliaceae	*Liliummartagon*		High
	Lilium hybrids		
	Brunello (Oriental)	*Wilting, withering*	Low
	Montanegro (Oriental)		Non-sensitive
	Star gazer (Asiatic)		Moderate
	Woodraff memory (Asiatic)		Low/non sensitive
Plumbaginaceae	Limonium latifolium		Very high sensitive
Rosaceae	Rose	*Abnormal flower opening*	High sensitive

Reid and Dodge 2000; Reid 2004; Macnish et al. 2011.

Prevention of Negative Effect of Ethylene on Cut Flowers

Whether cut flowers are sensitive to ethylene or not is affected by ethylene. Therefore it is necessary to protect of flowers against to negative effect of ethylene. In previously studies, two strategy was developed, towards preventing or reducing of ethylene effects. While the first strategy is to prevent to ethylene biosynthesis, the other one is to inhibition of ethylene action**.**

Prevention of Ethylene Biosynthesis

The various chemicals are used for inhibition of endogenous ethylene production, and also delaying senescence of cut flowers. The use of inhibitors of ethylene biosynthesis leads to a reduction of the endogenous ethylene production of plants. Some of these inhibitors are: cobalt ions (Lau and Yang. 1976), aminoxyacetic acid (AOA, Baker et al., 1982), aminoethoxyvinylglycine (AVG, Yang and Hoffman 1984), and methoxvinylglycine (MVG, Reid and Wu 1992). Preparation of AVG and MVG of these chemical is to difficult, and it has been found expensive for practical use. The compounds like AOA and AVG even reduced endogenous ethylene production of flowers, they did not effect on exogenous ethylene that exposed of flowers during postharvest transportation, storage and marketing process.

Prevention of Ethylene Action

Because of the prevention of endogenous ethylene production of cut flower species can not be successful for extending of postharvest life of flowers; the studies has been directed preventing of negative effect of exogenous ethylene. Ethylene action inhibitors are interact with ethylene receptors, and reduced by response to ethylene (Scariot et al. 2014). These compounds are; silver thiosulphate (STS, Veen 1979), 2.5 norbornadiene (2.5 NBD, Wang and Woodson 1989), diazocyclopentadiene (DACP, Serek et al. 1994), and 1-methylcyclopropene (1-MCP, Serek et al., 2006).Silver thiosulphate (STS) has been used commercially in many species of cut flower's as an inhibitor of ethylene action (Veen 1983). Because silver contained in STS has environmentally pollutant effect, it is considered harmfull compound and leads to reducing of application value (Serek and Reid 1993).Silver thiosulphate (STS) has been used commercially in many species of cut flower's as an inhibitor of ethylene action (Veen 1983). Because silver contained in STS has environmentally pollutant effect, it is considered harmfull compound and leads to reducing of application value (Serek and Reid 1993). Results a series of studies examining structure of the binding site during the ethylene biosynthesis, a compound that ethylene action inhibitor is found named 2.5 norbornadiene (NBD) (Sisler and Blankenship 1993).2.5 NBD, however, has not been commercially implemented, because of the lack of permanent effect, toxic effects, and bad smell. Similarly, DACP has not been used due to explosive effect and not stable.1-MCP used as an inhibitor of ethylene action is a gaseous, non-toxic chemical that inhibited ethylene action due to binding to its receptor. Because of the 1-MCP is a gaseous form, difficulties in practical application has encountered (Serek et al. 2006).Thus, in latest studies, a formulation of as a non-volatile form 1-MCP in the structure of nanosponge-based β-cyclodexrin (β-CD-NS, 1:8) has been developed (Seglie et al. 2011).

Ethylene Sensitive Flowers Species and Anti-Ethylene Treatments

Roses:The first negative effect of ethylene on cut rose flowers were determined by early flower-opening or early petal abscission due to excess ethylene given by lamps used for lighthing (Zimmerman 1931). Similarly, petal abscission of Sonia cut flower cultivar was accelerated with ethephone treatments (de Stigler 1981). The negative effects of exogenous ethylene (0.5 ppm) on cut rose flowers were defined as such as do not opening of flower bud or early flower opening, acceleration of petal abscission and the distortion of opened flower.

In all rose cultivars, however, sensitivity of ethylene was not found, and the effect of exogenous ethylene on opening of flowers and senescence changed by dependent on species (Oraee et al. 2011). When examined 38 different hybrid tea rose genotype, it was determined that the vase life of roses changed 4.5-18.8 day at 21oC, and also rate of opening of flowers were showed significant differences among the genotypes. When these genotypes treated with ethylene at the dose of 1µL/L (21^{o}C temperature 24 hours), 27 of these showed petal wiltering and abscision, and the vase life of these genotypes were decreased 0.8-84 day (%18-47) response to ethylene. In ethylene sensitive 17 genotypes, the rate of opening flower were decreased but the vase life of flower irresponsive to ethylene, and also 5 genotypes were not response to against ethylene (Macnish 2010). The various applications were performed to the ethylene-sensitive rose varieties for maintain of the negative effect of ethylene and improved of vase life.The oldest method of these applications is silverthiosulphate (GTS) addition into vase solution (Reid 2004). These treatments had been used in large quantities in initially, but it has lost its importance due to harmful effects of silver on the environment. In contrast, in the recent years in the limited rate of STS is used with 1-MCP, sucrose application, HQS, nitric oxid, thidiazuon, sodium benzoat for the increase of resistance and vase life of flowers (Table 2).

Table 2. The applications preventing ethylene effect on cut rose flower varieties.

Variety	Treatments	Dose/time	Effect	References
First red	STS	0.5 mM	Increasing of vase life	Chamani et al..,
Rosa hybrida L. cv. Diana	STS, Sucrose+HQS	0.2 mM , 2h STS +120g/L sucrose+10h HQS	Increasing of vase life to 9-10 days	Liao et al. 2000.
Golden Gate	1-MCP	0.4 µL/l/4 saat	Increasing of vase life	Philosoph-Hadas et al. 2005
Grand-Gala	Ethylbloc® (1-MCP)	1.5 g/m^3 ethylbloc®/ 3h	Increasing of vase life to 7.2 day	Da Rocha Batista et al. 2009.
Konfeti			Increasing of flower life (12.1 day) and leaf life (10.8 day).	
Rosa hybrida cv. ‘Sensiro	Nitric Oxid (NO)	40µL/L	Delaying of ethylene production,extending of vase life, delaying of flower senescence.	Mortazavi et al. 2010
	Thidiazuron (TDZ)	40µL/L		
	Nitric oxid (NO)+Thidiazuron (TDZ)	40+40µL/L		
Rosa hybrida L. cv. Avalanche	Sodium Benzoat	250 mg/L	Increasing of vase life, decreasing of ethylene production.	Imani et al., 2012
Amada	STS	0.5 mM /2 saat	Extending of vase life, maintaning of natural appearance, delaying of senescence.	Asghari et al. 2014

STS: Silverthiosulphate; 1-MCP: 1-methylcyclopropene, HQS: Hydroxyquinolinesulphate

Carnations;Ethylene production of carnations is increased during senescence and there is climacteric ethylene rise in petals (Woodson et al. 1992). Carnations species, however, showed sensitivity against exogenous ethylene, and exposure to ethylene causes premature petal wilting, referred as "Sleepiness". Some of the newer cultivars are less sensitive to ethylene than the standard ‘Sim’ types, and carnations have now been genetically modified by

the addition of a mutation of the ethylene binding site that makes them insensitive to ethylene (Reid 1997).Postharvest studies for the protection of the negative effects of ethylene to ethylene sensitive carnation varieties was began in 1980's, however, innovative studies is still ongoing with emerging technologies (Table 3).

Table 3. The applications preventing ethylene effect on cut carnation flower varieties.

Variety	Treatment	Dose/Time	Effect	References
--	Aminooxyacetic acid (AOA)	100 mM/10 min.	Delaying of climacteric ethylene rise.	Fujino et al., 1980
	Etanol	%1-%8	Suppressing of ethylene production, extending vase life.	Pun et al. 1999).
Dianthus caryophyllus cv. Barbara		%1 v/v	Delaying petal senescence, increasing vase life	Pun et al., 2014
	2,5 Norbornadiene	1000µL/L	Preventing ethylene binding (41%)	Sisler et. al., 1986
	Silverthiosulphate (GTS)	4 mM	Preventing ethylene binding (81%)	Sisler et. al., 1986
	B-Cyclodextrin based nanosponge and complex of 1-MCP (β-NS+1-MCP)	0.25 µL/L	Decreasing ethylene production, retaining petal color, preventing senescence	Seglie et al., 2011
Yelllow Candy	1-MCP	0.2-1 µL/L/20 saat	Blocking ethylene effect, incresing flower quality	Philosoph-Hadas et al. 2005

Lisianthus: Lisianthus is one of the flower species that sensitive to ethylene. Flowers of this species ethylene causes premature senescence. Therefore, the presence of ethylene into flower environment is the most important factor that limiting vase life. Lisianthus flower is produced ethylene especially pistil during flower development, while ethylene sensitivity can not observe during anthezis, sensitivity increased with senescence (Ichimura et al. 1998). Thus, for the reducing of ethylene-sensitivity of flowers; STS (Cho et al. 2001), 1-MCP (500 nL/L)+holding solution (6% sucrose+glucose, Cho et al. 2001), ethanol treatment (25-50 kg/L; Farokhzad et al. 2005), benzyladenine (BA; Hassanpour-Asil and Karimi, 2010) or asetaldehyde (1, 2, and 4%; Seighalani et al. 2013) is used.

Anthirhinum: Although snapdragon flower is preferred because of spike-type flower structure and colors, vase life of flowers is relatively short due to ethylene sensitivity (Serek et al., 1995). The effect of ethylene show itself especially on florets on the spike. But, ethylene sensitivity varies according to variety, and in some snapdragon variety there is natural resistance to ethylene. Whereas, especially susceptible varieties should treated with ethylene action inhibitor 1-MCP or STS.Thus, it was determined that in Rocket and Potamac Pink snapdragon varieties since 1 µL/L ethylene caused 100% floret abscission, it was determined that using of STS or 1-MCP to preventing negative effect of ethylene is necessary (Çelik et al. 2010).

Freesia: Ethylene sensitivity of freesia varies as depends on opening or non-opening of florets. While opened florets on the spike were not affect from ethylene, ethylene is prevented of development of unopened florets, thereby vase life of freesia flower is shorten. Therefore the use of ethylene action inhibitor compound is required to increase the postharvest vase life of freesia. It is found that one of this compounds, STS was effective to prevent floret abortion on the spike (Reid, 2000). Also, vase life of spike was increased using of 1-MCP (4nL/L; 4h) (Zencirkıran 2005).

Lilies: Flower life of lilium is an important quality criteria as in other type flowers. In general, vase life of lily varied from 5 days to 14 days depends on variety and postharvest handling process, and vase life is ended with wilting or withering of petals. It is determined that, lilies were much less sensitive to ethylene; susceptibility is varies according to variety (Woltering and van Doorn 1998); also vase life of flower is decreased by ethylene (Jones and Moody1993).Lily plants are divided into three main groups regarding ethylene susceptibility.(1) Unaffected by ethylene exposure.; The varieties of oriental lily and Lilium longiflorum are not affected by ethylene.(2) Minor responses to ethylene; some Asian varieties (Apeldoorn, Goldena, Elite, Mona) is effected from ethylene a little rate.(3) The effect of ethylene depends on concentration; ethylene at the high concentration decreased open-flower rate and opened-bud rate in the Asian lily varieties like Cordeila and Preta.Even ethylene reduced opened-bud or opened-flower, it did not effect on flower quality. Therefore, STS and 1-MCP treatments did not provide succeed against protect from ethylene, but vase life of 1- MCP treated Elite and Cordelia varieties was increased about 1.5 day (Elgar et al. 1999).

Gypsophila: Gypsophila is an important cut flower species is used as filling floral arrangements. However, postharvest vase life of flower is short due to unsuitable handling conditions and especially ethylene. Vase life of flower is reduced by early senescence caused by increasing of exogenous ethylene (van Doorn and Reid, 1992). While ethylene caused by wilting to opened flower, it is caused sleep or sleepiness or do not open, in buds. STS (4mmol/L, 30 min.; van Doorn and Reid, 1992) and AVG were tested for eliminate the negative effect of ethylene on gypsophila flowers, but both chemical compounds did not provide much more protective effect against exogenous ethylene. Therefore, 1-MCP that developed as alternative to STS was protect opened-flowers to ethylene effect but then it lost its effectiveness on newly opened flowers (Newman et al. 1998).

Orchids: Orchid flowers are extremely sensitive to ethylene at a very low level (Goh et al., 1985). Ethylene production rate of flower increased by pollination and emasculation, and result fast wilting. In the orchids species like Cymbidium, dortitaenopsis, dendrobium and plahaenopsis flower wilting is accelerated as a result of ethylene production following pollination; water loss occurs from cells on the outside of petals; the petals are folded down, and it becomes water soaked appearance (Porat 1994). AOA application to orchids as an ethylene biosynthesis inhibitor are not reduce ethylene sensitivity (Wang and Wang 2000). But, vase life of Oncidium Goldiana is increased by STS treatment at 30 min (Ong and Lim 1983); and similarly STS treatments at the doses of 4mM for 10 min is increased vase life of flower (Hew et al. 1983). The harmful effect of ethylene on Dendrobium and phalaenopsis flowers was inhibited completely with STS treatment (0.5 mM, 24 h) following 1-MCP treatment (0.1µL/L, 12h) (Wang and Wang 2000). Although 1-MCP treatment (0.1-0.4 µL/L) has protective effect to high concentration of ethylene (10 µL/L); its effect did not continue more than 7 days, at room temperature. In phalaenopsis cut flower trade, flowers is treated with 0.5 mM STS after cut, immediately.

Other flowers:Phlox (*Phlox paniculata* cv. Rambrant) flowers are one of the most sensitive species to ethylene. Exposure to ethylene enhanced flower abscission in a concentration-dependent (1 µL/L ethylene, 12 h; 50% abscission). It also accelerates the opening of ethylene flowers. The use of 1-methylcyclopropene (l-MCP), completely inhibited the ethylene-induced flower abscission and hence the reduction in the number of open flowers on the stems.l-MCP was maximally effective in inhibiting the ethylene response at a low concentration (25 nl/L) (Porat et al. 1995). Ethylene also accelerates petal abscission of Dahlia flowers. Ethylene production of pistil and petals during harvest is relatively high. 1-MCP and 5-benzylaminopurine (BA) applications are extended the vase life of flowers (Shimizu Yumoto and Ichimura 2013).In campanula varieties using as cut flowers like *C.*

barbata L., *C. latifolia* L., *and C. apunculoides* or *C. thacheliu*, 1-MCP application (1.14 pL / L) reduces senescence. C. barbata from these varieties gave the best results in terms of both increasing flower life and maintaining the petal color (Scariot et al., 2008).

Conclusion

Maintaining of quality of the cut flowers is the most important issues for both florist and dealing with flowers on the world trade. therefore, researchers are studied in terms of application for delaying senescence also increasing vase life. Flower senescence and vase life of flower are determined by so many factors. One of these factors, and perhaps most important one is ethylene that both produced by flower, and by ethylene supply into flowers environment. Ethylene, a plant hormone that is naturally produced during the growth of the cut flower, also limits postharvest life of plants. Ethylene caused: yellowing of leaf, abscission of petals, irregular flower opening, early flower death. Therefore, studies that determined the effect of ethylene on flower and prevention of ethylene effect were important. In this study, sensitiviy of different cut flower to ethylene and the methods to prevent ethylene effects were investigated. The negative effect of ethylene varies according to species, ethylene amount that exposure flower, and maturity stages of flower. Hence, it must be determined that the response of cut flower to ethylene, and the applicaiton must be done based on species and development stages of flowers.

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THE EFFECTS OF THE BIOLOGICAL ACTIVE FORMULATES AND STRAINS AGAINST THE CROWN ROT (Phytophthora cactorum)

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Abstract

Crown rot is one of the most common plant pathogen, especially temperate climatic zones, and can infect more than 200 species, including the apple tree (*Malus domestica* Borkh.). It is often observed as the crown, collar and root rot in the apple trees. Although some control strategies are commonly recommended to control the pathogen, such as using resistant varieties or using fungicides, new and innovative approaches have promising results that can use in the near future, such as biological active formulations (BAF) and strains.

Efficacy of 10 BAF and strains (Companion, Symbion Vam, Green Miracle, Cropset, Isr-2000, Actinovate, Subtilex, Tricho plus, Combat Plus, Endo Roots Souble) against the crown rot on the Red Chief variety grafted onto MM106 rootstock were examined in the screen house for 4 months. The soil mixture artificially infected with the pathogen isolated from naturally infected apple trees was used at the experiments. Treatments were applied to the plantlets with 3 replications and each replicate consisted of the 6 plantlets that were transplanted the soil and BAFs and strains were treated in the soil, and the biological parameters of the plantlets, trunk diameter, tree height, branch lengths and branch number, were observed during the experiments. Among the BAFs and strains, Actinovate was the most effective products.

Keywords: *Crown rot, Apple, Biological active formulations*

Introduction

Apple is a widely cultivated plant species in different ecological conditions of Turkey and the World. Turkey is the third leading producer, accounting for 3128450 tonnes of world production (Anonymous, 2015). The crown rot caused by *Phytophthora cactorum* (Lebert & John) Schröeter is an important disease of the apple trees. Phytophthora root and crown rots (sometimes called collar rot) are common and destructive diseases of fruit trees throughout the World (Jeffers and Wilcox 1990; Brewster et al. 1997). Maden et al. (1995) and Kurbetli and Değirmenci (2011) have reported that crown rot is the most common plant pathogen in apple orchard fields and plantlet production fields where it resulted in severe yield reduction and decline of growth.

The trees infected by crown rot are commonly found in poorly drained areas of the orchard or yard. Heavy and wet soils that remain saturated for extended periods of time are required for disease development. Above-ground symptoms vary between tree species, but generally include reduced vigour and growth of the plant, yellowing or chlorosis of leaves, and eventual collapse or death of the tree. Rapid death of trees usually occurs following excessively wet periods. On trees that decline gradually, a reddish or purple discoloration of the leaves often occurs in autumn, while leaves on healthy trees remain green. Below-ground symptoms include reddish-brown discolouration of the inner bark and wood. A sharp line shows the contrast between the infected and the healthy portions of the crown (Watkins et al., 1992;

Ellis, 2008; Nakova, 2010). Literature reviews show that the members of Phytophthora are widely spread on fruit trees (apples, cherries, apricots, peaches and almonds), citrus trees, forest trees, ornamental trees and other species. (Erwin and Riberio, 2005).

Controlling Phytophthora crown and root rot can be successfully done using an integrated program of cultural practices, like choosing the most resistant tree species or rootstock, and using fungicides if it is necessary. Technical, economic and environmental factors are forcing to adopt new sustainable methods, such as the use of microbial antagonists, to control soil borne pathogens. Researches, have mainly focused on antagonistic fungi and bacteria, demonstrated that using these type of biologic Plant Protection Products (PPP) don't give promising results. Therefore, another new approach has investigated against to plant pathogens, combination of the biocontrol agents and some factors (agro-chemicals, agronomical treatments, solarisation or steam sterilization, enhancement of suppressive soils, amendment of the soil. Various biocontrol strategies were developed for different pathogens (Spadaro and Gullino, 2005).

Soil consist of many type of microorganism, and one of them is called as Plant Growth Promoting Rhizobacteria (PGPR) related to growth of plant root. Some of PGPR might act as bio-control agent or plant growth regulator in plants and improve plant growth. (Altın and Bora, 2005; Ganeshan and Kumar, 2005). PGPRs can impoverish harmful organism via antibiotics secreted root zone or improve systemic acquired resistance (Turan et al., 2014). Biological agents involved in eleven products were evaluated with a plant growth assay in pot using plantlets of MM106 rootstock to evaluate their effectiveness in controlling *Phythophtora cactorum*. The trial was performed with soil artificially inoculated with the pathogen.

Materials and Methods

Crownt rot isolates used in this study were isolated from naturally infected apple trees from Isparta. *P. cactorum* was placed and sub-cultured on carrot agar and the petri dishes were incubated at 23°C for 7 days. A few agar plug of *P. cactorum* were transferred in jars that a mixture containing volumes of inoculum (400 ml carrot juice + 560 ml vermiculite + 40 ml the seed of the oat cultures) and incubated for 14 days at 23°C until colony growth covered entire inoculum mixture. The inoculum mixture was added to soil mixture (1:1 mixture of garden soil: sand). The same soil mixture amended so that 3% of the final volume was a 14 day-old inoculum mixture (40:56:4, vol/vol, carrot broth: vermiculite: oat medium at 23°C) (Latorre et al., 2001).

In experiment, Red Chief variety grafted onto MM106 rootstock was used and treated ten active ingredient/microorganism for *P. cactorum.* Recommended ratio of all products are used in the trial. Alexin 95 PS formulates was used to compare other bio-formulates (Table 1). Plantlets were transplanted to a 7 litre pot containing to an infested soil mixture. Treatments were applied to plants with 3 replications and every replicate was 6 plants. Plants were placed in screen house for 4 months, and were kept constantly wet. Pots were subjected them in dripping at 6 h. three times during week. At the end of the growing season, trunk diameter (10 cm above the graft union), tree height, branch number (> 20 cm/tree), and individual branch lengths were recorded for all trees in all experiments. The results were subjected to analysis of variance in SAS-JUMP 7.0.

Table 1. The List of Product of Bio-Formulates.

Name and Action	Active Ingredient/Microorganism	Company or Origin
Companion	*Bacillus subtilis* GB03	Growth products
Symbion Vam	*Glomus Fasciculatum*	Stanes
Green Miracle	*Vegetable oil acid*	Stanes
Cropset	*Lactobacillus acidophilus, plant extract, MnSO4, FeSO4, CuSO4*	Ant tarım (Improcrop EU)
Isr-2000	*Lactobacillus acidophilus, plant extract, yeast extract, benzoic acid*	Ant tarım (Improcrop EU)
Actinovate	*Streptomyces lydicus*	Mts agro
Subtilex	*Bacillus subtilis* MBI 600	Bioglobal
Tricho plus	*Trichoderma harzihanum*	Bioglobal
Alexin 95 PS	*Fosfor pentaoksin (P2O5) 52%, Potasyum Oksit (K2O) 42%*	Sumitoma
Combat Plus	*Plant activators*	Bioglobal
Endo Roots Souble	*Glomus intraradices.....21* *Glomus aggregatum.....20* *Glomus mosseage.....20* *Glomus clarum.....1* *Glomus monosporus.....1* *Glomus deserticola.....1* *Glomus brasilianum.....1* *Glomus etunicatum.....1* *Gigaspora margarita.....1*	Bioglobal

Results and Discussion

At the end of the growing season, trunk diameter (10 cm above the graft union) were recorded for all trees in all experiments. Trunk diameters of the plants were near each other and the differences between them were not found significant statistically (Table 2). Individual branch lengths (>20 cm) were recorded for all trees in all experiments and found unimportant statistically. The differences between branch numbers were found important statistically. The most effective formulates against crown rot disease were ISR-2000 (2.4), Alexin 95 PS (2.3), K0 (2.2) and the least effective formulates were determined as Tricho Plus (1.1), Symbion Vam (1.4), and K1 (1.5), respectively. There were severe differences between trees heights, and these disparities were statistically important. The most effective formulates were found as Cropset (107.2), Green Miracle (105.2), Actinovate (102.3), Alexin 95PS (102.3) while the least effective treatments were Tricho Plus (83.1) and K1 (88.7), respectively.

The effect of fungicides on mycelial growth of crown rot has been reported in many studies (Weiland et al. 2009; Boughalleb et al. 2006; Thomidis and Tsipouridis 2001; Utkhede and Smith 1991; Tewoldemedhin et al.2011; Kurbetli et al. 2015). Biocontrol of *Pythium* and *Phytophthora* species with *Enterobacter cloacae* and *Erwinia herbicola* (Nelson 1988), *Enterobacter aerogenes* and *Bacillus subtilis* (Gupta and Utkhede 1986), *Trichoderma* (Roiger and Jeffer 1991), and *Muscodor albus* (Mercier and Manker 2005) have also been reported. It is well known that many *Trichoderma* and *Bacillus* species are suppressive to

plant pathogens in soil (Papavizas, 1985; Weller, 1988). For this reason, the effect of bio-fumigation on beneficial microorganisms should be the topic of further studies.

Table 2. Efficacy of the BAFs and strains on the biological parameters of the plant infected by crown rot

Product	Trunk Diameter	Tree high	Branch length	Branch number
Actinovate	12.1^{NS}	102,3 ab*	38,4 ^{NS}	1,7bcd*
Alexin 95 PS	12.1	102,3 ab	33,1	2,3ab
Combat Plus	12.5	99,7 ab	36,3	1,6cd
Companion	12.2	100,1 ab	39,9	2,0abc
Cropset	13.0	107,2 a	36,3	2,1abc
Endo Roots Souble	12.7	100,1 ab	35,9	1,9abc
Green Miracle	13.1	105,2 ab	39,5	1,9abc
Isr-2000	13.0	100,3 ab	35,7	2,4a*
K0	13.0	100,3 ab	34,2	2,2abc
K1	12.9	88,7 cd	32,6	1,5cd
Subtilex	12.9	100,6 ab	34,5	1,8abc
Symbion Vam	12.8	95,6 bc	38,9	1,4cd
Tricho Plus	12.0	83,1 d	35,5	1,1d

^{NS} ; Not significant. There was no significant differences between the averages in the same column ($P>0.05$). *: Different letters indicate significant differences among means within a column ($P\leq0.05$) according to LSD Test.

Correa et al. (2010) has found that the target actinomycete strains are able to improve plant growth and nutrition, and benefit root colonization by AM fungi. Co-inoculation with both types of microorganisms showed synergic effects at enhancing plant growth and nutrient acquisition. Mohandas et al. (2013) has shown that it is clearly indicated the possibilities of using mycorrhizae associated actinomycetes as bioinoculant for growth promotion, nutrient mobilization and biocontrol agent in guava seedling production. Some biocontrol agents (*S. griseus, S. lydicus, B. subtilis* and *T. harzianum*) could be a possible alternative control measures of tea root disease, which in turn reduces the residual risk in made tea and it totally improves the tea ecosystem (Elango et al. 2015). Our finding are in agreement with the previous studies that they have shown that rhizosphere bacterial communities may suppress root infection caused by soil-borne pathogens. For example, inoculation with an antagonistic Bacillus subtilis strain reportedly protected young apple trees from *Phytophthora cactorum* crown and root rot (Utkhede et al., 2001).

Conclusion

As a result, the studies that we have done on crown rot have shown that some BAFs and strains, such as Cropset, ISR-2000, Green Miracle, Actinovate, Alexin can be successfully used against the crown rot. Further studies are required to investigate whether plant activators and biocontrol agents are compatible and can give enhanced control *of Phytophthora* in the field when applied alone or in combination.

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FIRST REPORT OF WHITE MOLD OF THE GLOBE ARTICHOKE CAUSED BY SCLEROTINIA SCLEROTIORUM IN TURKEY

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Abstract

During the growing season 2013-2014, symptoms of severe leaf wilting and necrosis, brown to black buds and dark brown xylem discoloration were observed on globe artichoke [*Cynara cardunculus* var. *scolymus* (L.). Fiori] plants from a field in Cesme, Izmir province, Turkey. The plants were stunted and had water-soaked rotting of stems, smaller edible buds which were discolored with dried outer bracts, the roots exhibited vascular discoloration as previously described in Italy and Peru. Infected stem and root tissues collected from the field were surface disinfested in a 0,5 % NaOCl solution for 90 s, rinsed with sterile distilled water, and air dried before plating on potato dextrose agar (PDA). White mycelium and black sclerotia (2 to 3 mm in diameter and 4 to 7 mm long), characteristic of *Sclerotinia sclerotiorum* (Lib.) de Bary, were produced in all dishes. Identity of the pathogen was confirmed based on colony morphology and formation of sclerotia. Koch's postulates were fulfilled using a 2-day-old *S. sclerotiorum* colony growing on PDA, root dip inoculation technique. Three days after inoculation, all seedlings, inoculated with *S. sclerotiorum*, wilted and expressed symptoms similar to those observed in the field. None of the control plants showed symptoms of infection. Two weeks after inoculation, the pathogen was successfully reisolated onto PDA. To our knowledge, this is the first report of white mold caused by *Sclerotinia sclerotiorum* on globe artichoke in Turkey. Since Izmir is the largest cultivator of globe artichoke in Turkey, it is an important step to manage white mold in this crop.

Key words: Globe artichoke, fungal diseases, white mold, *Sclerotinia sclerotiorum*

Introduction

Globe artichoke is an ingredient of the Mediterranean food, which is also used for the production of medicines and dietary supplements. This plant is known for its therapeutic potential as diuretic, antidiabetic and antimicrobial agent. More than 130 000 ha of globe artichokes are cultivated in more than 30 countries worldwide. In terms of total production area (2 680,2 ha) and total production amount (34 576 tonnes) Turkey is one of the world's important producers of globe artichoke (Anonymous, 2015). Diseases cause significant economic losses to globe artichoke production. *Sclerotinia sclerotiorum* (Lib.) de Bary is a fungal pathogen causing white mold disease in a wide range of plants. It is capable of colonizing over 400 plant species found worldwide. *S. sclerotiorum* is an ascomycetous necrotroph fungus dispersed as airborne ascospores or soilborne sclerotia. The fungus can grow well even in an unfavorable environment and survive for up to 8 years in soil in the sclerotia form (Adams and Ayers, 1979). It causes water-soaked lesions on the leaves or stem rot in stems of some infected plants. The most obvious symptoms of plants infected by *S. sclerotiorum* are necrotic tissues covered with patches of fluffy white mycelia. Sclerotia are produced after mycelial growth when the nutrition is not sufficient or other conditions are favourable for sclerotial development (Christias and Lockwood, 1973). They play an important role in disease cycles as they are the primary structures for their long-term survival and produce inocula for further infection. Sizes of sclerotia are dramatically different

depending on their host (Wang et al., 2008). This study confirms the presence of the white mould, caused by *S. sclerotiorum*, on globe artichoke in Turkey.

Materials and Methods

During the growing season 2013-2014, *Sclerotinia sclerotiorum* isolates collected from globe artichoke production areas, in Cesme, Izmir province, western Turkey were used in this study. Infected plants were stunted and had water-soaked rotting of stems, smaller edible buds which were discolored with dried outer bracts, the roots exhibited vascular discoloration (Figure 1 and Figure 2).

(A) (B)

Fig.1. Dark brown xylem discoloration in transverse (A), and longitudinal (B) sections of roots of white mold–affected globe artichokes.

(A) (B) (C)

Fig. 2. Foliar symptoms of white mold in globe artichoke; Severe leaf wilting and necrosis (A), brown to black bud (B) and dark brown xylem (C).

The causal agent was isolated from symptomatic stems and roots. Small pieces of diseased stem and root tissues collected from the field were on the surface sterilized in a 0,5 % NaOCl solution for 90 s, rinsed with sterile distilled water, and air dried before plating on PDA. Inoculated plates were incubated at 22 ± 2°C. Identity of the pathogen was confirmed based on colony morphology and formation of sclerotia. Fungus was purified and microscopic characterization was done according to Bolton et al. (2006). The cultures were stored on PDA slopes at 4 °C and subcultured every 6 months. Koch's postulates were fulfilled using a 2-day-old *S. sclerotiorum* colony growing on PDA, root dip inoculation technique (Del Río et al., 2005; Bhat et al., 1999).

Results and Discussion

All tested isolates caused typical symptoms on globe artichoke of white mold. Inoculated plants developed symptoms of wilting 3 days after inoculation and were completely wilted within 15 to 20 days. Symptoms of wilting were soon followed by the appearance of white mycelium and black sclerotia. The control plants didn't exhibit symptoms and *Sclerotinia sclerotiorum* was reisolated from inoculated plants in two weeks (Figure 3). A fungus was consistently isolated from symptomatic stems of globe artichokes. The isolates showed specificity being pathogenic to the host they were obtained from.

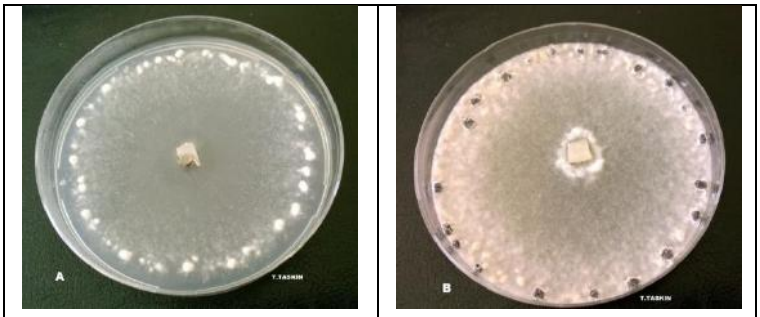

Fig.3. White mycelium (A) and black sclerotia (B) of a culture of *Sclerotinia sclerotiorum* isolated from infected globe artichoke growing on PDA.

To our knowledge, this is the first report of white mold, caused by *Sclerotinia sclerotiorum* on globe artichoke in Turkey. The identification of globe artichoke as a host for *S. sclerotiorum* is consistent with earlier researches. *Sclerotinia sclerotiorum* may occur with wide distribution in fields where very little or none had occurred in the previous year; the occurrence of *S. sclerotiorum* is associated with prolonged wet weather that is favorable for production of apothecia (Abawi and Grogan, 1979). When environmental conditions are suitable, copious amounts of white cottony mycelium and the subsequent production of black sclerotia of variable size and shape which are signs of the fungus occur. This pathogen is geographically cosmopolitan and has a broad ecological distribution, although it is most common in temperate regions. This disease was also reported on globe artichoke in Italy and also in Peru (Marcucci et al., 2010; Tarazona Matos, 2009).

Conclusion

Sclerotinia sclerotiorum is one of the important plant pathogen. White mold is a very serious problem in crops, especially when they are cultivated in contaminated wet soils in cool and wet weather. Extensive crop damage, lack of high levels of host resistance, and the general difficulty of managing diseases caused by *Sclerotinia* have been the impetus for sustainable research on this pathogen. This study is an important step to perform practical and cost-efficient strategies for managing white mold, which is an important effect limiting globe artichoke production. Further studies are needed to determine the distribution of white mold on globe artichoke in Turkey. Management of *Sclerotinia sclerotiorum* occurs at several stages of crop development. Techniques include: making the crop microclimate less conducive for infection, utilization of effective fungicides to protect susceptible plants, elimination of sources of inoculum, and selection of resistant plants. Successful disease control commonly requires implementation and integration of multiple techniques.

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DIAGNOSIS OF SOME SEED-BORNE FUNGI ON SOME BARLEY GENETIC SOURCES IN YEMEN

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Abstract

Given the important economic losses caused by the seed-borne fungi to yield barley crop, with the absence of previous studies in Yemen by this study aimed to isolate and diagnosis the fungi. A laboratory study was carried out to investigate the seed-borne fungi and their effect on seed germination and seedlings growth of 64 sources gene of barley from the germplasm center of the Agricultural Research and Extension Authority (AREA),Yemen. Tested methods: incubation on blotting paper and on the Potato Dextrose Agar media (PDA) was proven that all the seeds in the study were carriers of a number of fungi, was recorded 7th genus of the fungus transmitted on the seed sources of barley according to the following: *Fusarium* spp, *Drechslera* spp, *Alternaria* sp, *Cladosporium* sp, *Ustilago* spp, *Aspergillus* sp and *Rhizopus* sp, and the results showed that there were differences between the genetic resources of barley on the percentage of the presence of fungus on various sources, they ranged between 3.3% -50%, and the *Fusarium* spp more genus fungal isolates in frequency and to cause a rot of the seeds and the death of seedlings, the source of genetic YG: 20217 More devoid of seed borne fungi, while the source of genetic YG: 20016 more sources borne fungi.

Key words: *seed-borne, fungi, barley genetic, Yemen*

Introduction

Barley is one of the important cereal crops on a number of the world's countries , and using seeds as a highly nutritional crops for human and animal feed, or as materials fodder green and may enter the barley with forage material mixture of leguminous crops like *Medicago* spp. and cowpea fodder to improve the nutritional value of the material feed while barley is used in Yemen as food for humans where it enters in many local meals and work Loaf (Almiloj) and local bread (Mujahid,1986).Barley occupies the fourth place among the world's cereal crops in terms of cultivated area and average production and is grown in most temperate regions and tropical under which distributed in the cultivation of wheat and barley, in Yemen is considered the most important field crops that are spread grown in cold areas of the central highlands and occupy an important position both in rain-fed or irrigated agricultural systems(Mujahid,1986).

Barley cultivation in Yemen suffer a severe decline in the productivity per unit area, where agricultural statistics show since 2000 and until 2008, the average productivity of grain ranged between 0.622 - 1.297 t / h , compared with the global average for the production of barley Ranged between 2.086 - 3.009 t / h for the same period (FAO, 2010).

The low crop yields per unit area return to several reasons, including the infecting of pests, from which the pathogenic fungus that is transmitted by seeds which cause economic losses starting with 4% and the passage of the seasons and frequency of cultivation the infected seeds that cause increased of economic loss all season even up to 80% of the yield, some fungi infect the crop in the field, such as fungus *Fusarium* , there is also other fungi movement with grain from the field to the store and causing discoloration of the seed ,

damage and the deterioration of their quality when the availability of conditions during storage period, such as *Penicillium*, *Aspergillus*, *Alternaria* and *Rhizopus* (Yahyaoui et al, 2004).

Given the important economic losses caused by these seed-borne fungi to yield barley crop, with the absence of previous studies in Yemen by this study aimed to isolate and diagnosis seed-borne fungi from some genetic barley sources collected from different regions of the Republic of Yemen and studied its effect on germination and infecting of seedling rates.

Materials and methods

The study was conducted on 64 seeds genetically source of barley, which have been obtained from the Genetic Resources Center for Agricultural Research and Extension Authority (AREA) of the Republic of Yemen.

Germination percentage test & Isolation of fungi

The following methods used to germination percentage test and isolation fungi from the barley seeds:

Planting in wet blotting paper

Wrapped blotting scale 24.5 cm rolls opposite paper so that the paper contains grooves where seeds are placed, put the leaves in a rectangular plastic dishes sterilized previously by alcohol ethanol concentration of 70%.To sterilize the seeds: 30^{th} seed from each genetic source immerse in Sodium hypo chlorite solution concentration of 1% for a period of 5 -3 minutes and then the seeds was tacked out and submerged in sterile and distilled water for 3-2 minutes to remove the sterile solution and after dried and placed in the grooves blotting paper at a rate of 10 seeds in each groove at a rate of three replicates each genetic source, put plastic dishes in a room temperature 25 c under the lighting 12 hours a day for a week, taking into account the daily moisturizing seeds sterile and distilled water, and after the completion of the germination process are recorded number of seeds , germinating seeds, rotting seeds and calculate the percentage of each genetic source , after that the isolation from rotting seeds was done .

Cultured way

The culture medium potato dextrose agar (PDA) was prepared and added the antibiotic Chloramphenicol at a rate of 0.05 g /L to prevent the growth of bacteria. It cleared 20 seed each genetic source superficial immerses for 3 minutes with a solution of sodium hypochlorite concentration of 1%. Seeds are sown on cultured PDA at 5 seeds in each Petri dish diameter of 15 cm and incubated dishes at a temperature of 2 ± 25 c under the lighting 12 hours a day reciprocal with the dark for a week and then examined colonies fungal formed on the seed and quoted all fungi to the culture medium new developing seeds Each genetic source separately in Petri dishes 9 cm in diameter were incubated in the previous incubation conditions for its development in the pure culture, was diagnosed depending on the form of the fungal colonies and the conidia and conidiophore of fungal spores and using some of classification keys of fungi (HASHMI & GHAFFAR. 2006 , GOEL & AULAKH. 1980.).

Results and discussion

The impact of fungus transmitted by seed of barley genetic resources Table 1 shows the results of impact seed- born fungi on the seed germination rates and found that the germination ranged between 20% -90% rates of the injury seeds fungi accompaniment ratios between 3.3% -50% and this is due to moisture high availability method of culture conditions in the paper in which the grew wet especially fungi because it helps speed the germination of spores and growth of fungal mycelium on seeds and this in turn led to affect seed germination percentage (SIMARD and LUDWIG, 1948). A negative correlation have found ($r = -0.541$) between the percentage of germination and the percentage of rot seeds , so when the rot seeds

increased the germination will decreased and this causes losses to the farmer consumes of seeds not only had a low germination rates, but requires to replanting the fail lines of farm and the cost of agriculture must be increased, these findings are consistent with a grandfather researchers. (IT and Somerx'ille, 1997, MAMLUK & VAN LEUR. 1986, SIMARD and LUDWIG, 1948.) , and found that the genetic source YG: 20217 more sources sensitivity where the percentage of germination and rot seeds 20 and 80%, respectively, in While the genetic source YG: 20016 more bearable sources where the percentage of germination and seed rot amounted to 90% & 10% respectively

Table (1) Effect of seed- born fungi on the germination and rot of the genetic sources of Yemen’s barley seeds

No.	Input code	germinated seeds	germinated seeds%	seeds rotting	seeds rotting %
1	YG:20016	27.0	90.0	3	10.0
2	YG:20035	24.0	80.0	6	20.0
3	YG:20048	22.0	73.3	8	26.7
4	YG:20049	19.0	63.3	11	36.7
5	YG:20053	14.0	46.7	16	53.3
6	YG:20055	16.0	53.3	14	46.7
7	YG:20056	10.0	33.3	20	66.7
8	YG:20057	26.0	86.7	4	10
9	YG:20059	20.0	66.7	10	33.3
10	YG:20089	13.0	43.3	17	56.7
11	YG:20090	14.0	46.7	16	53.3
12	1YG:2009	11.0	36.7	19	63.3
13	YG:20095	18.0	60.0	12	40.0
14	YG:20103	16.0	53.3	14	46.7
15	YG:20110	17.0	56.7	13	43.3
16	1YG:2011	13.0	43.3	17	56.7
17	YG:20112	17.0	56.7	13	43.3
18	YG:20114	25.0	83.3	5	16.7
19	YG:20115	18.0	60.0	12	40.0
20	YG:20116	18.0	60.0	12	40.0
21	YG:20124	23.0	76.7	7	23.3
22	YG:20125	18.0	60.0	12	40.0
23	YG:20128	15.0	50.0	15	50.0
24	YG:20129	23.0	76.7	7	23.3
25	YG:20130	16.0	53.3	14	46.7
26	1YG:2013	22.0	73.3	8	26.7
27	YG:20155	25.0	83.3	5	16.7
28	YG:20156	21.0	70.0	9	30.0
29	YG:20157	24.0	80.0	6	20.0
30	YG:20159	19.0	63.3	11	36.7
31	YG:20177	25.0	83.3	5	16.7
32	YG:20179	21.0	70.0	9	30.0
33	1YG:2018	17.0	56.7	13	43.3
34	YG:20182	23.0	76.7	7	23.3
35	YG:20183	23.0	76.7	7	23.3
36	YG:20184	21.0	70.0	9	30.0

37	YG:20187	19.0	63.3	11	36.7
38	YG:20188	18.0	60.0	12	40.0
39	YG:20189	15.0	50.0	15	50.0
40	YG:20192	17.0	56.7	13	43.3
41	YG:20195	25.0	83.3	5	16.7

Continued Table (1) Effect of seed- born fungi on the germination and rot of the genetic sources of Yemen’s barley seeds

No	Input Code	seeds germinating	seeds germinating%	seeds rotting	seeds rotting%
42	YG:20198	22.0	73.3	8	26.7
43	YG:20199	13.0	43.3	17	56.7
44	YG:20202	17.0	56.7	13	43.3
45	YG:20203	20.0	66.7	10	33.3
46	YG:20206	16.0	53.3	14	46.7
47	YG:20211	10.0	33.3	20	66.7
48	YG:20212	10.0	33.3	20	66.7
49	YG:20213	15.0	50.0	15	50.0
50	YG:20214	23.0	76.7	7	23.3
51	YG:20215	9.0	30.0	21	70.0
52	YG:20216	17.0	56.7	13	43.3
53	YG:20217	6.0	20.0	24	80.0
54	YG:20218	15.0	50.0	15	50.0
55	YG:20219	17.0	56.7	13	43.3
56	YG:20221	22.0	73.3	8	26.7
57	YG:20224	18.0	60.0	12	40.0
58	YG:20225	23.0	76.7	7	23.3
59	YG:20229	20.0	66.7	10	33.3
60	YG:20230	17.0	56.7	13	43.3
61	YG:20233	16.0	53.3	14	46.7
62	YG:20236	19.0	63.3	11	36.7
63	YG:20243	19.0	63.3	10	33.3
64	YG:20247	19.0	63.3	11	36.7

The isolate and diagnose fungi associated with the seeds of barley genetic resources results indicated in Table 2 that the isolated result showed seven genus of fungi associated with the seeds of barley genetic resources are: *Fusarium* spp, *Drechslera* spp, *Alternaria* sp, *Cladosporium* sp, *Ustilago* spp, *Aspergillus* sp, *Rhizopus* sp. The genus *Fusarium* sp. (Image 1) was the most fungi infected the seed sources genetic barley may be is causing the rot of most seeds studied, and causing delay of germination, while the genus *Rhizopus* sp. Which the less fungi reluctant to most seed sources barley Genetic , these findings are consistent with the findings of researchers (ABUGHNIA and FARAJ, 1978, IT and Somerx'ille, 1997, Yahyaoui et al., 2004).

Indicated precedent Research (Malone and Lorimer, 1975) that the cover of the seed is the houses of the fungus cell, where it is the fungi *Rhizopus* sp, *Aspergillus* sp. present on the surface in the casing not affect vital seeds which fungi store, and this explains the high germination genetic source 6 YG: 2001 with the infected by the genus *Rhizopus* sp.(IT and Somerx'ille, 1997, Yahyaoui et al., 2004) while the cover and inside the seed houses of the following fungi *Fusarium* spp, *Drechslera* spp, *Alternaria* sp, *Cladosporium* sp, *Ustilago* spp

This fungi transmitted from the field and remain static in the seed until the next season and do more harm to their impact on the vitality of seeds germination or kill the seeds in addition to other fungi of rot seed when the availability of appropriate conditions because of enzymes degrading and toxins produced by fungi that affect the seeds and this explains the low germination of the genetic source YG: 20217 because of this injury of the fungi *Fusarium* spp, *Drechslera* spp, *Alternaria* sp respectively These findings are consistent with what the researchers. (Rao et al., 1999, LEE. 1980, IT and Somerx'ille, 1997, Hashmi and Ghaffar , 2006, Mamluk and van Leur, 1986, BEARDALL & MILLER. 1994.)

It also found fungi nurse moves from the last season by seeds such as *Fusarium* fungus that causes the disease blight seedling and symptoms in the field seedling dwarf yellow and then die, *Drechslera* fungus which causes rot disease seedling and symptoms in the field, brown spots. The fungus *Alternaria*, which causes blight grain and symptoms in the field, the presence of black dots on grain. The fungus *Ustilago* which causes covered -smut and symptoms disease in mass field of fungus spores replace and covered grains. This is the first study refers to isolate and diagnose fungi associated with barley seeds in Yemen and our results are compatible with what the researchers (Rao et al., 1999, IT and Somerx'ille, 1997, Lee, 1980) indicated previous studies (Lee, 1980, Goel and Aulakh, 1980) that the losses caused by the previous diseases mentioned on the barley crop, which is the loss accumulated increase from last season planting infected grain and so any attempt to improve barley in these affected areas will be failed unless the farmars must be use a health and sterilized by fungicides seeds.

Table (2) Seed-born fungi transmitted by seeds of barley of Yemen’s genetic sources.

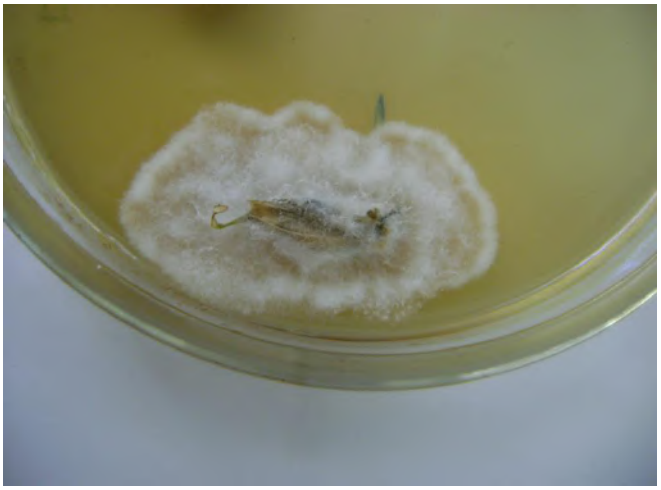
No	Input Code	*Fusarium* spp	*Ustilago* spp	*Rhizopus* sp	*Alternaria* sp	*Cladosporium* sp	*Aspergillus* sp
1	YG:20016	-	-	+	-	-	-
2	YG:20035	-	-	-	-	-	+
3	YG:20048	+	-	-	+	-	-
4	YG:20049	+	-	-	-	-	-
5	YG:20053	+	+	-	-	-	-
6	YG:20055	-	-	+	-	-	+
7	YG:20056	+	+	-	-	-	-
8	YG:20057	+	+	-	-	-	-
9	YG:20059	+	-	-	-	+	+
10	YG:20089	-	-	-	-	-	+
11	YG:20090	+	-	-	-	+	-
12	YG:20091	+	-	-	-	-	-
13	YG:20095	+	-	-	-	-	-
14	YG:20103	+	-	-	-	+	-
15	YG:20110	+	-	-	-	-	+
16	YG:20111	+	-	-	-	-	-
17	YG:20112	+	+	-	-	-	+
18	YG:20114	+	-	-	-	-	+
19	YG:20115	+	+	-	-	-	-
20	YG:20116	+	+	-	-	-	-
21	YG:20124	+	-	-	-	-	-
22	YG:20125	+	+	-	-	-	-
23	YG:20128	+	-	+	+	-	+
24	YG:20129	+	-	-	-	-	-

25	YG:20130	+	-	-	-	+	-
26	YG:20131	+	+	-	-	-	-
27	YG:20155	+	-	-	-	+	-
28	YG:20156	+	+	-	-	-	-
29	YG:20157	+	-	-	+	+	-
30	YG:20159	+	+	-	-	-	+
31	YG:20177	+	-	-	+	-	-
32	YG:20179	+	-	-	-	-	+
33	YG:20181	+	-	-	-	-	+
34	YG:20182	-	-	-	-	+	+
35	YG:20183	+	-	-	-	+	+
36	YG:20184	+	-	-	-	-	+
37	YG:20187	+	-	-	-	+	-
38	YG:20188	+	-	-	-	-	-
39	YG:20189	+	-	-	-	+	-
40	YG:20192	+	-	-	+	+	-
41	YG:20195	+	+	-	-	+	-

Continued Table (2) Seed-born fungi transmitted by seeds of barley of Yemen’s genetic sources.

No	Input Code	*Fusarium* spp	*Ustilago* spp	*Rhizopus* sp	*Alternaria* sp	*Cladosporium* sp	*Aspergillus* sp
42	YG:20198	+	+	-	+	-	-
43	YG:20199	+	+	-	+	-	-
44	YG:20202	+	-	-	-	-	+
45	YG:20203	+	+	-	+	+	+
46	YG:20206	-	-	+	+	-	+
47	YG:20211	+	-	+	+	-	-
48	YG:20212	+	-	-	-	-	-
49	YG:20213	+	-	-	+	-	-
50	YG:20214	+	-	-	-	-	+
51	YG:20215	+	-	-	-	-	-
52	YG:20216	+	-	-	-	-	-
53	YG:20217	+	-	-	+	-	-
54	YG:20218	+	-	-	+	+	+
55	YG:20219	+	-	-	-	-	+
56	YG:20221	+	-	-	-	-	+
57	YG:20224	+	+	-	-	+	-
58	YG:20225	+	-	-	-	+	-
59	YG:20229	+	+	-	-	+	-
60	YG:20230	+	-	-	-	+	-
61	YG:20233	+	-	-	+	-	-
62	YG:20236	+	-	-	-	+	-
63	YG:20243	+	-	-	-	+	-
64	YG:20247	+	+	-	-	+	+

Alternaria sp



Fusarium spp

Image (1) some seed born fungi on the seeds of barley genetic sources of Yemen

Conclusions

The study was recorded 7th genus of the fungus transmitted by the seed sources of barley: *Fusarium* spp, *Drechslera* spp, *Alternaria* sp, *Cladosporium* sp, *Ustilago* spp, *Aspergillus* sp and *Rhizopus* sp. The seed-borne fungi caused an economic losses to yield of barley crop, so the main recommendations of this study are: The seeds must be testing for the seed-born fungi before entering in the gene bank , keeping under cooling and fungicide treatment. Also the re-planting seeds annually to maintain the vitality is very important to conservation of the genetic sources of the Yemeni's barley.

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ZEARALENON CONTAMINATION IN CORN FOR FOOD AND FEED IN REPUBLIC OF SRPSKA

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Abstract

This study was a survey of estrogenic mycotoxin zearalenone contamination in corn for human consumption and feed. Samples were taken by the representatives of the Agricultural Extension Service of Republic of Srpska from field or different storage facilities in regions of Banja Luka, Prijedor, Doboj, Bijeljina and Gradiška, where corn is commonly grown. Samples are analyzed by competitive enzyme immunoassay (ELISA) with microplate based kit produced by EuroProxima, the Netherlands that also contains standards with different zearalenon concentration. In total, 77 samples are analyzed and the results showed that 58 samples (75,3%) were contaminated in a range from 100 to 986 μg/kg (ppb). Avarage content in all analyzed samples was 280,9 μg/kg.

Key words: *zearalenone, corn, Republic of Srpska.*

Introduction

Mycotoxins are secondary metabolites of fungi and that can develop on plants in the field and during storage. They are potentially toxic to humans and animals. Mycotoxins and mycotoxicosis were and still are a problem, although a scientific interest in mycotoxins began in the early 1960s. Increased interest in food safety in the whole world has influenced in increased interest of fungi contamination and mycotoxin production in foods. Mycotoxins commonly enter the food chain directly through contaminated food and indirectly through food of animal origin. Mycotoxins in foodstuffs appear in small doses, but its continuous intake in organism, even in a microdose, can result in their accumulation in human organism. In this sense , attention is continuously focused on *corn* (*Zea mays* L.) because it is one of the most important food and feed in different regions of the world (*Anon, 2004*). Corn is the third most important crop in the world after rice and wheat In the Republic of Srpska corn is mostly grown for grain and it takes almost a quarter of the total arable landin the country.

In recent years there are significant climatic disturbances at the global level accompanied by extremely high or low temperatures, emergence of large and heavy rains with floods or droughts occurrences. These stressful conditions have largely contributed the contamination of different cultivars of cereals with fungi and their metabolites, mycotoxins. In the Republic of Srpska the previous, 2014, was almost ideal (especially due to favorable weather conditions) for development of the *Fusarium* species which attack maize at the time of maturity.

Zearalenone (ZON) is a natural contaminant of harvested and stored grain. They are a metabolites of 18 species of the genus *Fusarium* (*F. graminearum, F. sporotrichioides, F. semitectum, F. equiseti, F. crookwellense, F. culmorum,* etc.) and include 15 derivatives of zearalenone with estrogenic effects. These mycotoxins are known for oestrogenic and anabolic effects on humans and animals. Consumption of large doses of ZON in food may cause the effect of food refusal, sexual apathy, miscarriages and cancer effects (carcinoma of the prostate for men and cervical cancerin for women). This is because ZON is quickly absorbed through intestinal tract. In children population it causes a syndrome of premature

puberty. ZON concentration of 60 μM for 72 h inhibits over 80% cell growth, DNA and protein synthesis (Abid-Esefi *et al.*, 2004). The aim of this study was to monitor the presence and distribution, as well the concentration of zearalenone in corn produced in the Republic of Srpska intended for human and animal nutrition.

Materials and Methods

In total 77 samples of corn intended for human consumption and for animal feed is analyzed. Samples were taken by the representatives of the Agricultural Extension Service of Republic of Srpska from field or different storage facilities in regions of Banja Luka, Prijedor, Doboj, Bijeljina and Gradiška, where corn is mostly grown. Samples were analyzed in Laboratory for Plant Protection of Agricultural Institute of Republic of Srpska, Banja Luka.

The quantity of 250 g of each sample was dried at 60°C for 72 h and in this way samples were prepared for further analysis on mycotoxins presence. Approximately 50-100 g of each sample was grinded and homogenized to a fine powder and 5ml of PBS/methanol was added to the samples in the proportions 40:60. Prepared samples were stirred for 30 min and centrifuged for 10 min at 2000 xg. The quantity of 25 μl of the supernatant obtained after centrifugation was diluted with 150 μl of dilution buffer. For further testing is used 50 μl of the dilutions, and each sample was tested in two replicates.

Samples were analyzed by competitive enzyme immunoassay (ELISA) with microplate based kit produced by „Euro Proxima“ (the Netherlands) that also contains standards with different zearalenon concentration. The ELISA test is highly sensitive and specific immunological reaction which enables the detection and quantification of very low concentrations of zearalenone. Test is based on the antibody for zearalenone. The microtiter plate is coated with sheep antibodies for rabbit IgG. Antibodies (rebbit anti-zearalenone), enzyme-labeled zearalenone (enzyme conjugate) and zearalenone standards or samples are added simultaneously to the pre-coated wells of a microtiter plate, and then incubated for 1 hour at 37°C. After incubation the enzyme labeled reagents are removed by washing. The amount of bound enzyme conjugate is visualized by adding of substrate chromogen (tetramethylbenzidine, TMB). The bound enzyme is transformed into stained chromogen product (Fig. 1). The substrate reaction was stopped by the adding of sulfuric acid. The mean optical density (OD) is measured photometrically at 450 nm and is inversely proportional to the concentration of zearalenone in the sample.

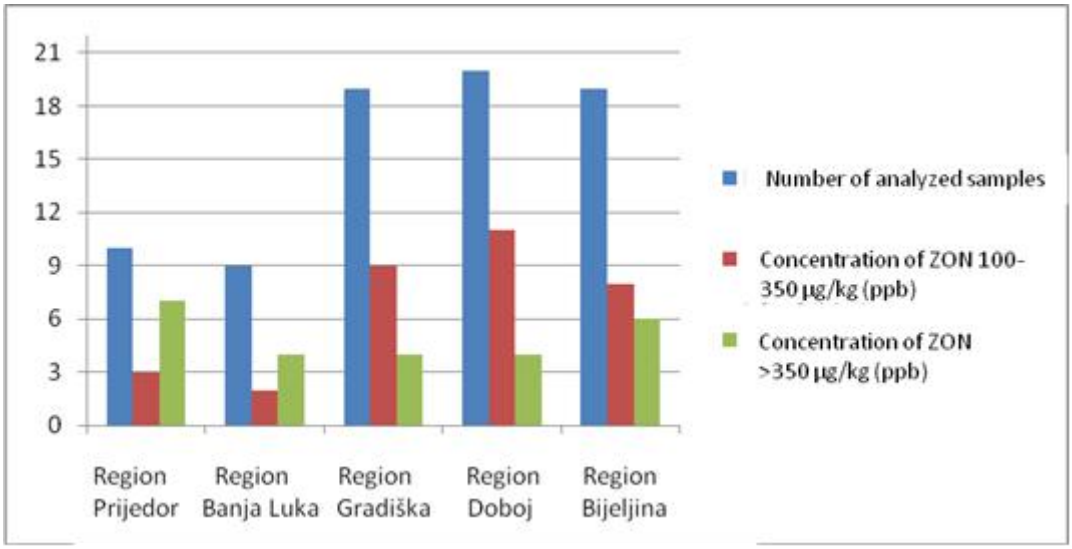
Photometric measurements were carried out on the Thermo Scientific™ Multiskan™ FC microplate photometer. The mean optical density (OD) of blank wells is subtracted from individual OD values of wells containing standards and samples. The OD value of six standards and the samples (value of the duplicates) are devided by the mean OD value of zero standard (wells A1 and A2) and multiplied by 100. Values (% maximum absorbance) calculated for the standards are plotted on the Y axis versus the zearalenone equivalents concentration (ng/ml) on a logaritimic X-axis. The amount of zearalenone in the samples is expressed as zearalenone equivalents. The zearalenone equivalents in the extracts (ng/ml) corresponding to the % maximal absorbance of each extract can be read from the calibration curve. Calculated equivalents are multiplied by a factor 120. All these calculations were done using the Thermo Scientific™ SkanIt™ software.

Results and Discussion

Results of analyzed samples of corn from different regions of the Republic of Srpska showed contamination levels of 75.3% or 75.3% while in samples was determined zearalenone content in the range from 100-986 ppb. Contamination of zearalenone in the amount of 100-350 μg/kg (ppb) were found in 42.8% of the analyzed samples, while in 32.5% of the analyzed samples zearalenone content was over 350 μg/kg (Table 1, graph.1).

Table 1. Presence of zearalenone in analyzed samples in examined regions

Region	Number of analzyed samples	Concentracion of ZON 100-350 µg/kg (ppb)	Concentracion of ZON >350 µg/kg (ppb)
Region Prijedor	**10**	**3**	**7**
Region Banja Luka	**9**	**2**	**4**
Region Gradiška	**19**	**9**	**4**
Region Doboj	**20**	**11**	**4**
Region Bijeljina	**19**	**8**	**6**
IN TOTAL:	**77**	**33**	**25**
%		**42,8**	**32,5**



Graph 1. Graphic presentation of results by regions.

The most contaminated samples were detected in region of Prijedor where the content of zearalenone was over 100 ppb in all 10 analyzed samples. In other regions, the percentage of contaminated samples ranged from 66% (Banja Luka Region) to 75% (Doboj Region). According to Regulations on maximum levels for certain contaminants in foods (Official Gazette Bosnia and Herzegovina 68/14) corn containing zearalenone between 100-350 µg/kg can not be used for direct human consumption, corn snacks and breakfast cereals. The average content of zearalenone in all analyzed samples was 280,9 µg/kg. In Bosnia and Herzegovina content of zearalenone in animal feed is not regulated. However, the introduction of zearalenone in the food chain through animal products has a huge importance, or when the animals eat contaminated food. If zearalenone is present in very high concentrations in animal feed, the risk of human exposure to this mycotoxin is high.

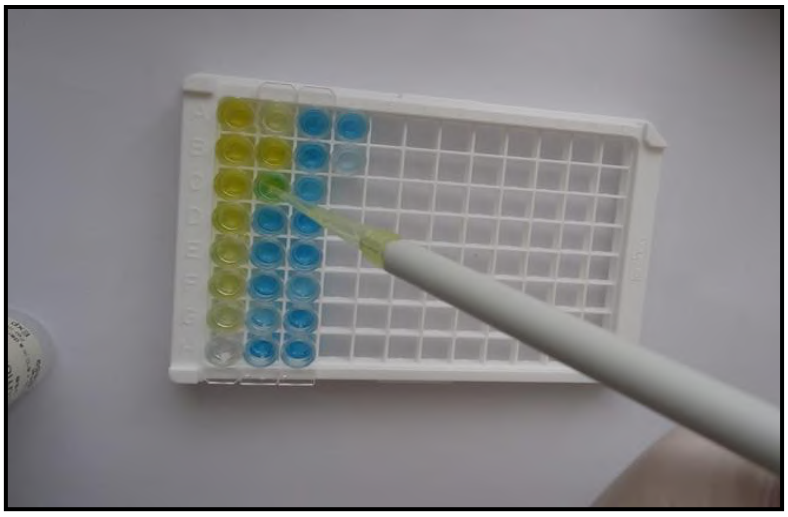


Fig. 1. Adding a chromogen substrate and transformation in the stained product

Mycotoxin zearalenone was named by the phytopathogenic fungus *Giberella* from which was isolated in 1962 (Delaš, 2010). The presence of zearalenone has been detected in various grains and corn, barley, wheat, rye, soybeans and their products (Ožegović and Pepeljnjak, 1995; Mitak, 1998; Duraković and Duraković, 2000). It is also found in milk, muscles, organs, tissues and eggs of animals that have been fed contaminated food (Prelusky *et al*., 1990). The available data in Europe show that corn is usually contaminated with zearalenone, but also a high content of zearalenone may have wheat, rye, oat and soy products (EC, 2004). Zearalenone is only partially decomposed under the influence of heat. Almost 60% of zearalenone in average remains unchanged in bread, while about 50% "survive" the production of macaroni. Concentration of zearalenone in fractions of flour and semolina is reduced by 80-90% during the dry milling of corn, while in the bran and germ found increased concentrations.

It is necessary to implement measures to minimize contamination of corn and other products during and after harvest, during processing and storage. Also, it is necessary to continue and expand the monitoring, as well to determine e relationship between this mycotoxin and health problems in humans.

Climatic conditions have a key role for production of mycotoxins. The ability of fungi to produce mycotoxins largely depends on temperature, relative humidity and stressful conditions to which plants are exposed (Miraglia *et al*., 2009). For all these reasons, further research of presence of mycotoxins and toxigenic fungi and their epidemiological importance in corn crops have a great importance.

Conclusions

Based on presented results it can be concluded that the climatic conditions in the Republic of Srpska during 2014 were extremly favorable for the appearance of mycotoxin zearalenone or fungi of *Fusarium* species which synthesize this toxin. Results also indicated a high level of contamination of unprocessed maize with mycotoxin zearalenone. Considering that the *Fusarium* species produce several types of toxins is would be reccomended to include other toxins such as fumonisin and deoxynivalenol, as well for other cereals (wheat, barley, and others.). In addition, it would be necessary to implement constant monitoring of imported food and feed for the presence of mycotoxins.

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EFFICIANCY AND SELECTIVITY OF SOME HERBICIDES AT SWEETCORN

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Abstract

During the period 2011 - 2014 years in the experimental field of Agricultural University – Plovdiv a study for researching the effect of various herbicides on the productivity of six hybrids sweet corn - Challenger (F1), Erica (F1), Vega (F1), Honey Bentam (F1), GSS (F1) and Denitza (F1) was conducted. The effect of various herbicides and their combinations on the yield of cobs was treced. From all the used herbicides and their combinations most favorable effect on the development and productivity was found when combination of herbicides Merlin Flex 480 SC + Laudis OD doses of 42g / da after sowing and before emergence for Merlin Flex and 200ml / da foliar application for Laudis was used, and this is statistically proven. When combination of Merlin Flex 480 SC + Laudis OD was used, suitable to realize the highest total yield of cobs is GSS hybrid, and highest percentage of the total standard cob yield was found at Chellenger and Denitza.

Key words: Sweet corn (Zea mays ssp. sacharata Sturt.), herbicides, hybrids, yield.

Introduction

(Zea mays var. Saccharata) is a subspecies of ordinary corn, and in recent years in Bulgaria couses considerable interest. A major problem in the cultivation of this culture is weeding. It leads to strong reduction in yield, especially in the high density of the weeds in the early stages - between 2nd and 4th leaf. Then the plants rely yield, and weeding in this period can even completely compromise the yield. When control the weeds by chemicals, a large number of hybrids exibit phytotoxicity, and the sweetcorn plants die (Sevov et al, 2014).

According Waligóra et al. (2012) herbicide combination foramsulfuron + iodosulfuron methylosodium, shows very good control of annual dicotyledonous weeds, disregarding phytotoxic effect on any of the tested hybrids. Pataky et al. (2008) have studied the sensitivity of the 149 hybrid sweet corn to the herbicide nicosulfuron, foramsulfuron and mesotrione. In comparative research with 95 hybrids, phytotoxicity which leads to a complete death of the culture was recorded. Odero et al. (2013), has found that the efficacy of herbicides pyroxasulfone, S-metolachlor and mesotrione on soils with a high organic content (over 85%) is excellent - from 91% to 99%, without adverse effect on the culture.

Kopsell et al. (2011); report for changes in of sunscreens carotenoids, β-carotene, chlorophyll A and B content as a result of foliar treatment with mesotrione + atrazine at Rugosa hybrid. Changsaluk et all, (2009) and Rajablarijani et al. (2014) have similar results for physiological changes due to different herbicides applying.

There are many data not only on the efficacy of different herbicides in the ordinary corn, but for their economic feasibility also (Koprivlenski, 1997; 2011), Modern research has shown that the new sweet corn hybrids are highly sensitive to some herbicides, but here the problem is poorly understood. Contemporary researches show that new sweet corn hybrids are highly sensitive to certain herbicides, but in our country this problem is poorly studied.

Material and methods

The research was conducted during the 2011 – 2013 period, in the experimental field of the Agricultural University – Plovdiv on alluvial soils. Randomized complete block design was

displayed with five variants in three replications and size of the experimental plot 20 m^2, (table 1). The efficacy and selectivity of 4 herbicides and herbicide combinations to 6 maize hybrids: Challanger F1, Erica F1, Vega F1, Honey Bentam F1, GSS F1 and Denitza F1 has been researched. Soil and foliar herbicides are imported with a knapsack sprayer and working solution 300-400 l / ha. During the vegetation drop irrigation of the crop has been done.

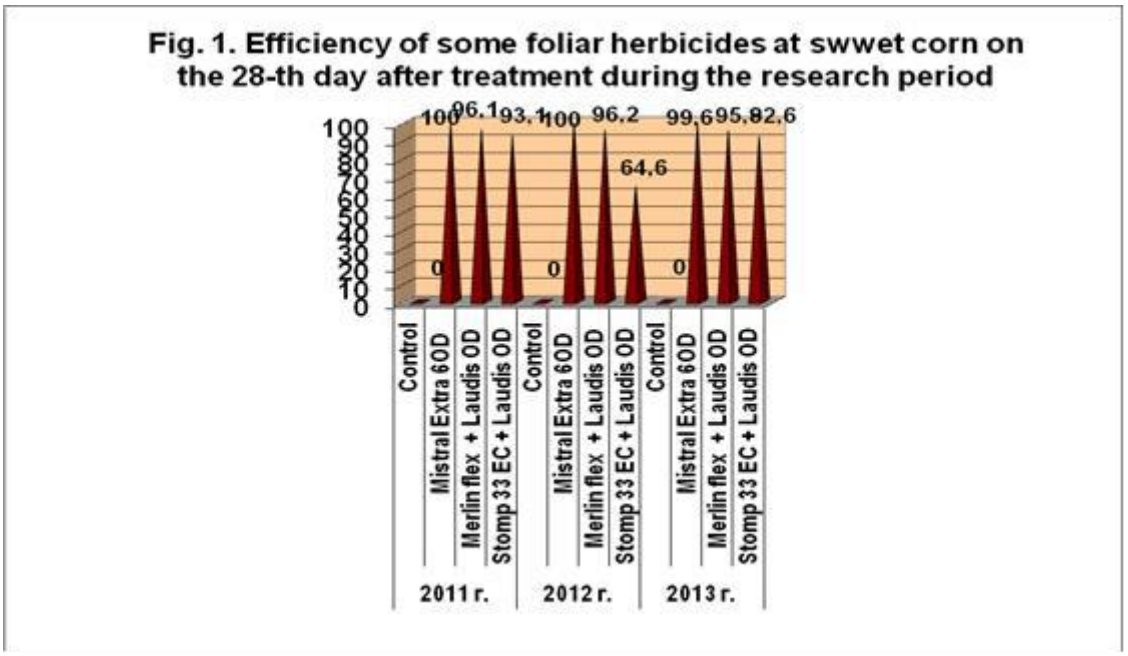
Table 1. Variants of experiment

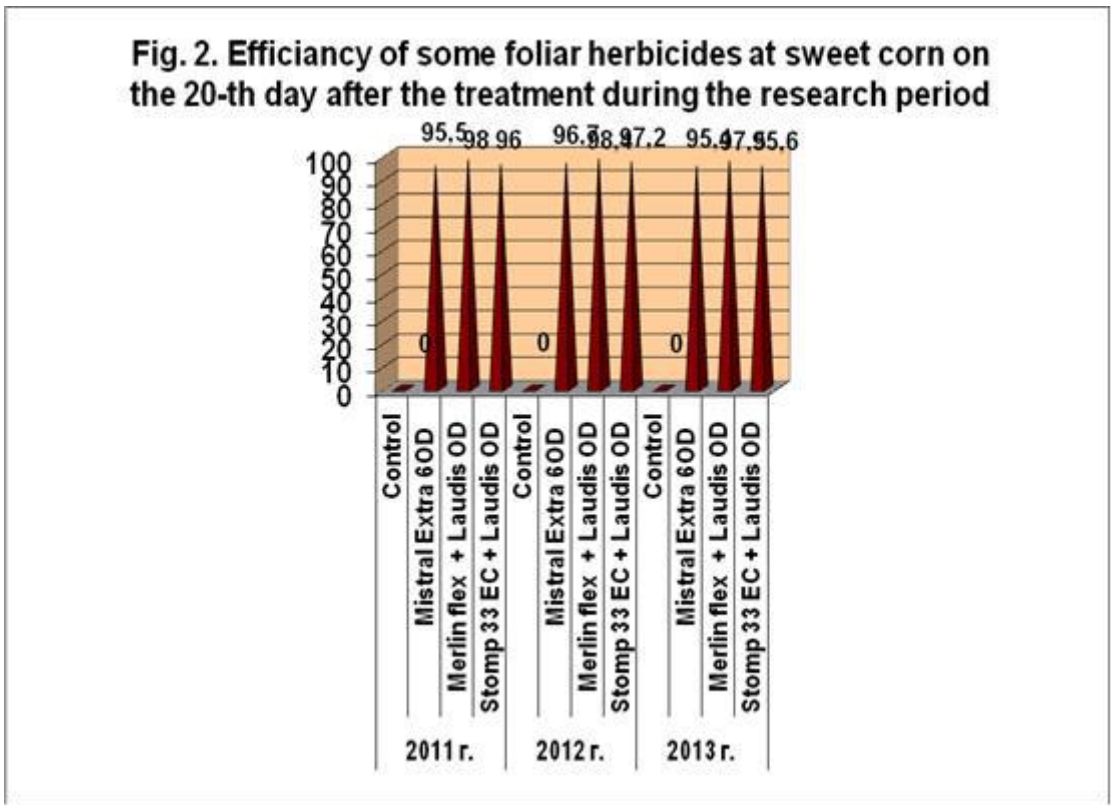
Variants	Herbicide	Active substance	Dose /ha
1	**Zero control (untreated)**	-	-
2	**Gardoprim Plus Gold 500 SC**	**312.5 g / l S-metolachlor + 187.5 g / l terbutilzin**	**4 l /ha** - *soil application*
3	**Mistral Extra 6OD**	**60g / l nicosulfuron**	**650 ml /ha** - *foliar application*
4	**Merlin flex + Laudis OD**	**240 g / l isoxaflutol + 44 g / l tembotrione**	**420 g /ha**- *soil application+* **2 l /ha**- *foliar application*
5	**Stomp 33 EC + Laudis OD**	**330 g / l pendimethalin + 44 g / l tembotrione**	**3,50 l /ha** - *soil application+* **2 l /ha**- *foliar application*

Years of the conducting experience are characterized with varying temperature and precipitation. The highest amount of precipitation in the period of sowing up to the 45^{th} day after treatment with soil herbicides (April and May) have fallen in 2013 - 183,0 mm, and at least in 2011 - 59,6 mm. In relation to temperature, warmest was 2013 - with temperature sum for April-May 34.0 C^0, and coolest is 2011 - 28,9S0.

Results and discussion

Sweet corn areas are weeding mainly with annual late spring weeds as: *Amaranthus retroflexus L., Solanum nigrum L., Chenopodium album L., Portulaca oleraceae L., Dathura stramonium L., Setaria viridis L., Setaria glauca L., Xanthium strumarium L., Echinochloa grus-galli L.*, rather than multi - *Sorghum halepense* L. In the untreated control the weed infestation level of is very high and ranges from 503 pcs. / m2 (2012) to 584 pcs / m2 (2013).
The efficacy of soil applied herbicides Gardoprim Plus Gold 500 SC, Merlin flex and Stomp new 330EK on the 28th day after treatment, average for the three year period is an excellent. In regard to the weeds highest efficiancy show Gardoprim Plus Gold 500 SC - 4 l / ha - 99,9%, followed by Merlin Flex - 4.2 l / ha - 96,1% and Stomp new 330EK - 3,5 l / ha (93,4%) (Figure 1).





Yearly, there was no substantial difference of the effect of the applied soil herbicides to the 45th day after treatment. Highest efficiency show again Gardoprim plus Gold 500 SC - 98.9%, followed by Merlin Flex - 4.2 l / ha - 93,7%. Less control on weeding - 89.7% was recorded in Stomp new 330EK - 3,5 l / ha.

For none of the used soil herbicides was established pytotoxity on the studied hybrids sweetcorn on the third, 7th and 21st day after the treatment (ball 1 on the scale of EWRS).

In foliar appended herbicides, both itself (var.3) and in combination with soil herbicides (var.4 and 5) efficacy on the 20th day after the treatment was very good.
In the variants where the combination Merlin Flex - 420 g / ha (soil application) + Laudis OD 2 l / ha- (foliar application) is applied the efficiency is 97.9%, and in variant 5 - Stomp new 33 EC - 3 5 l / ha (soil application) + Laudis OD - 2 l / ha (foliar application) - 96,3% (Figure 2). Lower weed control is obtained when treated with Mistral extra OD 6 - 650 ml / ha - 95,8%, and it is mainly due to weeds sprouted as a result of precipitation in April and May, and performed irrigations in the later period of vegetation of culture.
The herbicide controls the perenial species Sorghum halepense L. up to 99% and Laudis OD - 2 l / ha to 95% - 96%. In the untreated control the level of weed infestation is very high and ranges from 571 pcs. / M2 (2012) to 613 pcs. /m2 (2013).
Despite the high density of annual weeds in the research areas, the efficiency of the appended foliar herbicides, both alone and in combination is maintained until the 40th day after spraying.
In regard to the selectivity of the leaf herbicides negative impact on the studied hybrids sweetcorn is not reported, except Erica (F1). Mistral extra OD 6 at a dose 650 ml / ha and Laudis OD - 2 l / ha caused complete death of plants of this hybrid (ball 9 on the scale of EWRS). From 7 to 14 days after treatment, Laudis OD - 2 l / ha caused lightening of maize plants, which turn white from the top of the leaves to the base (ball 6-7 by EWRS) and on the 21th day wither and die (ball 9 on the scale of EWRS). When treated with Mistral Extra 6 OD on the 7th day, plants acquire anthocyanin colouring (ball 4 EWRS), on the day 14th necrosis (Ball 6 EWRS), and on the day 21st wither and die (9 ball EWRS).

Conclusions

Soil applied herbicides on the 28th day after treatment showed excellent effect against the weeds, which lasts until the 45th day after treatment. Efficacy varies from 93.4% at Stomp new 330EK - 3,5 l / ha, to 99.9% at Gardoprim plus Gold 500 SC - 4 l / ha.
Phytotoxicity of studied soil herbicides Gardoprim Plus Gold 500 SC at a dose of 4 l / ha, Merlin flex - 4,2 l / ha and Stomp new 330EK - 3,5 l / ha on the crop has not been established.

High efficacy against the weeds of leaf herbicides Extra Mistral OD 6 - 650 ml / ha - 95,8%, and the combination of Merlin Flex - 420 g / ha (soil +) Laudis OD 2 l / ha (leaf) - 97.9% has been reported.

Mistral Extra OD 6 - 650 ml / ha controls the perennial species Sorghum halepense L. up to 99% and Laudis OD - 2 l / ha up to 95% - 96%.
Prolonged control on weed infestation average of three years of investigations was reported when using combination of soil and foliar herbicide.
When treated with foliar herbicides Mistral 6 Extra OD at a dose of 650 ml / ha and Laudis OD - 2 l / ha phytotoxic effect on the hybrid Erica (F1) was reported and the herbicides cause complete death of plants - Ball 9 EWRS.

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COMPOSITION OF AROMA COMPOUNDS OF THE APPLE DISTILLATES FROM NATIVE VARIETIES OF NORTH GREECE

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Abstract

Apple (Malus domestica Borkh.) is one of the most widely cultivated fruit in Europe. The apple tree is a deciduous tree, in the rose family, it is cultivated in many geographical areas and is the most widely grown species in the genus malus. About 80 million tons of apples were grown world wide and are often eaten raw, bur also be used in agricultural industries in order to produce different products with important properties for human nutrition. Apple distillate or cider brandy, obtained by the distillation of cider, made from cider-apple cultivars, is a common drink in many areas of Europe. Apple distillates are made from apple pomace – the solid residues generated after juice extraction and is composed of peel, pulp and seeds. The aim of this study was to evaluate the chemical composition of cider brandies from different varieties cultured in Naoussa- Pilio of Continental Greece. Analysis of major volatie compounds were performed by Head Space GC-MS. In general 43 components were identified. Terpenes(geraniol, nerol, linalool, a-terpenol etc.,alcohols(hexanol, 2-phenyl-ethanol,1-propanol etc.,, aldehydes(acetaldehyde, acetal etc.), esters(isoamyl acetate, ethyl acetate,isoamyl cetate, ethyl lactate, ethyl isovalerate etc. and fatty acids (hexanoic acid, octanoic acid etc.) were the determined major components in our samples. The results indicated that cider brandies from native apple varieties from different cultured areas of continental Greece has low concentration of toxic methanol, and is considered a high quality product and a factor of important economic repercussion.

Key words: *Cider, Spirits, apples, volatiles, distillates*.

Introduction

The alcoholic beverage produced by distillation of fermented apple pomace it is the popular cider brandy. It is characterized by a special aromatic flavor. Some compounds of this beverage originate from the fruit variety, but most of them are developed during apple pomace fermentation. Various factors influence the aromatic profile such as cultivation environment, soil and climate, the apple variety, the degree of fruit ripeness, the distillation apparatus which used for the spirits production, the pomace fermentation conditions, the distillation process and the aging of the spirits, if is used .

Cider Brandy volatiles can be classified in different chemical groups such as alcohols, esters, aldehydes, ketones, acids, terpenes etc. (Guichard, H.et al.,2003)**.** These compounds can be characterized by different volatility. Some compounds highly volatile, whi1e others exhibit lower volatility. These aroma compounds exist in a wide concentration range. Some of them are present at high concentrations (hundreds of mg/L), but most of them are found very low concentration range, from traces to 10mg/L. The concentration of toxic methanol which is well-known that its levels of in alcoholic beverages are related with the pectic substances present in fruit, as well as to the presence of pectinmethylesterases with the capacity to degrade it. Methanol (CH_3OH, methyl alcohol,carbinol,wood spirit,wood alcohol) is a

colourless liquid at ambient temperatures, with a slight alcoholic pungent odour when pure. It is well known that methanol is a toxic and harmful substance to human health (when taken orally at 340 mg/kg of body weight) of whose ingestion or inhalation can cause blindness or death. Following ingestion, it is oxidised to formic acid, both toxic to the central nervous system. Formic aldehydes deteriorates the optical nerve, causing blindness and reported that oxidised methanol produces lactic acidosis which is a metabolic disease caused by an increase in blood levels of lactic acid. Its symptoms lead to weakness, vomiting and finally coma and death. The level of 200 mg/L is considered the maximum safe concentration to be inhaled for a continuous eight-hour working dayGerogiannaki- Christopoulou M. et al. 2007.
Furthermore, the higher alcohols formed from amino acids during fermentation are also affected by the yeast strains employed and the fermentation temperature of ciders. Acrolein,off-flavors in cider brandies could be formed during cider maturation by microorganisms (Mangas, J. J et al, 1996).
The aromatic profile of fermented beverages will especially mark the characteristics of commercial spirits; however, the chosen distillation system also plays an important role in the final composition of the distillate (Gerogiannaki- Christopoulou M., et al. 2007). For instance, the extended length of heating in distillation systems, produces cider brandies with higher contents in furfural due to degradation of residual sugars, thus imparting a caramel aroma. The composition of distillates likewise varies depending on the material employed in the construction of the distillation system. On the other hand, traditional practices in the production process increase consumer approval of products. Apple distillates produced in copper traditional distillation apparatus , which consist of a pear-shaped pot at the top coupled to a recipient filled with cool water for condensing the alcoholic vapor.
In this paper, we study the volatile compounds of apple distillates from different apple varieties after fermentation of apple pomace. To do so, we carried out a preliminary analysis of the chemical composition during the distillation process and subsequently performed a chemical evaluation of the cider spirits thus obtained. Analyses were carried out according to European Union regulation parameters (Council Regulation 110/2008.Volatile compounds were analyzed by Head –space GC-MS.

Materials and methods

Raw Material. Apple distillates from two apple varieties **(**Brina and Norfolk Royal) was studied**.** The spirits produced in local distilleries of North Greece by means of traditional technology and were prepared from the fermented apple pomace of monovarietal apples. The pomace of apples fermented by wild microflora.
Distillation. The distillation of apple fermented pomace were realized with the a traditional copper alambic of 130L. The copper alambic, is hermetically closed in order to prevent any vapor leakage. When the temperature reached at 85^0 C, the liquid spirit starts to run and collected.
The apple distillate sample, collected and analyzed by head space GC-MS.

Chemical Analysis.

Chemical Analysis of Volatile Compounds by Head Space GC-MS

The volatile components present in headspace fraction of distillates were isolated and identified by using a balance pressure headspace system Perkin-Elmer HS40 (Perkin-Elmer Analytical Instruments, Uberlingen, Germany) coupled to a GC/MS-Q 5050 system (Shimadzu Co, Kyoto, Japan). A 2 mL sample from each sample was taken and introduced into a 22 mL round-bottomed vial with 1 mL aliquot of standard solution (2 mg pentanol-3 in ethanol-water solution (50:50 v/v)); then, the vials were sealed with aluminium-rubber septa.

The vials with samples were held at 50 °C for 25 min, purged and pressurised with helium at a flow rate of 40 mL·min^{-1}. The volatile compounds were driven through the transfer line which was held at 80 °C to the injector of the gas chromatograph. The volatile compounds were separated on an HP Innowax capillary column (60 m length × 0.25 mm internal diameter, 0.25 μm film thickness) at the following conditions: injector temperature 200 °C; carrier gas helium 0.6 mL·min^{-1}; temperature program: 45-100 °C at a rate of 4 °C·min^{-1}, held for 5 min and go to 200 °C at a rate of 8 °C·min^{-1} and held for 12 min. The GC column was directly connected without splitting to the ion source of QP 5050 quadrupole mass spectrometric detector which was operating in the scan mode within a mass range of m/z 30-350 at 2 scans·s^{-1}. The interface line to MS was set at 250 °C. The MS was operating in an electron impact mode at electron energy of 70 eV and was calibrated by auto-tuning.
Identification of the compounds was carried out by computer-matching of their mass spectral data with those of known compounds in the Shimadzu NIST62 Mass spectral Database and by comparing their retention times and mass spectra to 3-pentanol as internal standards solution.

Quantification was performed by integrating the peak areas of TIC (total ion chromatograms) by the Shimadzu Class 500 software.

Oven temperature programme, 50-260 °C at a rate of 4 °C·min^{-1}; transfer line temperature, 270 °C; carrier gas, helium at a linear velocity of 31.5 cm·s^{-1}; inlet split ratio, 1:60; MS source ionization energy, 70 eV; scan time was 1 s, covering a mass range of 40-300 amu.
The constituents were identified by comparison of their mass spectra with those in a computer library (LIBR-TR and Wiley 5 Library) or with authentic compounds. The identifications were confirmed by comparison of their retention indices of volatiles either with those of authentic compounds or with data in the literature.
Quantitative results were obtained by calculating the average value of three samples.

Results and discussion

The distillation fractions were analyzed by Head space GC-MS using the method described above. Table 1 contains the identified components of apple distillate from the native apple varieties.A-pinene has the higher concentration (211μg/l) and methanol. (125mg/L), are the major compounds.
Methanol, a toxic alcohol is clearly below the maximum permissible limits (EC-110/2008).

Table 1: Experimental data for the concentration of volatiles constituents in cider brandies) from two apple varieties.

Apple distillate Volatile Compound				
	Brina Apple variety(Naouss)		**Norfolk Royal Apple variety(Pilio)**	
Terpenes and Norisoprenoids(μg/l)	**Terpenes and Norisoprenoids(μg/l)**		**Terpenes and Norisoprenoids(μg/l)**	
	Average	SD	Average	SD
Linalool	146	26	119	31
Nerol	167	22	128	19
Geraniol	64	7	57	
Citronellol	22	5	37	6
Citronellal	79	9	29	7
a-Pinene	211	19	241	22
b-Pinene	189	16	199	13
Theaspirane	30	5	46	6
a -Terpineol	31	4	45	
a- Ionone	9	3	7	4
Terpinen-4-ol	18	5	28	3

b- Ionone	56	11	32	9
p-Menth-1-en-7,8-diol	34	4	55	3
3-Oxo-a-ionol	138	17	103	19
4-Oxo-a-ionol	21	5	9	7
Alcohols(mg/l)				
		SD		SD
Methanol	125	5,1	119	7,2
1-Propanol	41	3,6	39	2,9
1-Butanol	9	1,9	6	1,5
2-Butanol	n.d		n.d.	
trans -2-Hexenol	0,04	2	0,2	1,5
1-Hexanol	2,6	1,1	1,9	0,9
3-Hexanol	26	4,7	29	3,2
trans -3-Hexenol	0,9	2,8	1,1	1,9
cis -3-Hexenol	1,1	2	2,4	2,1
Isobutanol	55	14	41	9
Isoamyl alcohols	189	21	125	17
2-Phenyl-ethanol	28	6	31	7
Benzyl alcohol	9	2,3	7	2,1
Esters				
Ethyl lactate	48	11	51	9
Ethyl acetate	119	23	156	26
Isobutyl acetate	29	9	32	11
Ethyl butyrate	45	12	49	8
Butyl acetate	13	6	19	7
Ethyl hexanoate	12	2,1	14	2,6
Ethyl octanoate	3,8	1,9	5,5	1,4
Ethyl decanoate	1,6	2	2,1	1,9
Ethyl dodecanoate	2,9	1	4,2	1,1
Diethyl succinate	1,1	2,3	1,9	2,7
Fatty acids				
Hexanoic acid	5,5	1,2	5,8	1,9
Octanoic acid	2,6	1,1	3,9	1,5
Decanoic acid	1,7	4	2,7	2,9
Other compounds				
Acetaldehyde	61	6	72	5
γ-Butyrolactone	2,2	3	3,4	2

Conclusion

In summary, apple distillates gives rise to spirits with aromatic character and the toxic methanol clearly below the maximum permissible limits. Furthermore, the levels for the studied aromatic volatiles are in consonance with those of other apple distillates.

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THE EFFECT OF MANURE, ZEOLITE AND MINERAL FERTILIZER ON THE YIELD AND MINERAL COMPOSITION OF CAULIFLOWER

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Abstract

In the present study the effect of various forms of fertilizers on the yield and mineral composition of cauliflower was examined. The experiment was conducted at the experimental field of University of Thessaly during 2013-2014 growing season. Four fertilizer treatments were applied: 1) Control (C), 2) farm yard manure (M), 3) standard fertilizer (F), 4) zeolite + standard fertilizer (F+Z). Seeds from cauliflower hybrid Centrum F1 were sown in seed trays containing peat and young seedlings were transplanted directly in the field. At the day of harvest plant features regarding plant development, such as the total plant fresh weight, the number of leaves, the fresh and dry weight of leaves, heads and shoots were recorded. In addition, mineral composition of heads and leaves were assessed. From the results it is suggested that the fertilizer type affects significantly the various plant features. More specifically, the treatments (F) and (F+Z) resulted in the highest total plant weight and head fresh weight, number of leaves and shoot weight and differed significantly from both control (C) and manure treatment (M). Regarding mineral composition, differences were observed in the Fe content of leaves, total nitrogen and Zn content of heads and leaves. In conclusion, the fertilizer type could affect both yield and mineral composition of cauliflower, whereas standard fertilizer application (alone or combined with zeolite) is essential for higher yield and final product quality.

Keywords: *cauliflower, fertlization, manure, nitrogen, zeolite*

Introduction

The use of chemical fertilizers is essential in order to achieve the maximum potential of the genetic material and, as well as to succeed the highest possible yield and quality of the final product. However, usually farmers are dependent on empirical methods for fertilizer application, without using the means that allow for better fertilizer use efficiency such as plant tissue and soil analysis. The common result is the irrational use of fertilizers that consequently leads to environmental pollution, and soil and ground water degradation, whereas sometimes such practices involve threats for human health due to low quality of the final product. The use of fertilizers other than chemical fertilizers (e.g. composts, manure, organic fertilizers) gradually gain interest as an environmentally friendly and compatible to sustainable agriculture practice (Knight and Newman, 2013). However, the main disadvantages of various organic fertilizers are the high cost per fertilizer unit due to limited nutrient availability, and the unstable composition, especially for products such as the manure.

Nitrogen is the one of the essential macronutrients that is important for plant growth and development and is widely added in crops in order to increase yield. Cekey et al. (2011) reported that increasing nitrogen rate in cauliflower crops up to 250 kg ha^{-1}, resulted in an increase of 31.4% in yield compared to control (no added fertilizers). Similarly, Bozkurt et al. (2011) reported that a nitrogen rate of 225 kg ha^{-1} combined with full irrigation treatment resulted in the highest yield for cauliflower crops. Quiros et al. (2014) examined the effect of three fertilizers (industrial and homemade compost and mineral fertilizer) on cauliflower crops. From the results it is concluded that although total yield was higher for mineral

fertilizer by 26 and 91% comparing to homemade and industrial compost respectively, the application of homemade compost had a significant effect on the quality of the final product, since the produced flower heads were bigger and heavier than the other treatments.
Zeolite has been widely used in agriculture for amelioration since it is suggested to improve soil properties, decrease nitrogen leaching and increase nitrogen recovery (Huang and Petrovic, 1994; Sepaskhah and Barzegar, 2010). Bybordi et al. (2013) evaluated the effect of zeolite and nitrogen application on plant growth, nitrate reductase activity and plant nutrient content of canola plants. From the results it was concluded that zeolite application can increase plant growth and seed production mostly due to better nitrogen use efficiency and soil amelioration. Similarly, the application of zeolite and urea resulted in an increase of dry matter content and mineral composition of silage corn (Bernardi et al., 2011).
The aim of the present study was to examine the effect of various fertilizers on plant growth and yield of cauliflower plants, as well as to evaluate the use of zeolite as a means for soil amendment and better use efficiency of nitrogen fertilizers.

Materials and Methods

The plant material used in the experiments was the cauliflower hybrid Centrum F1 [*Brassica oleracea* convar. *botrytis* (L.) Alef. var. *botrytis* L. (Rex F1)]. Seeds of the three species were sown in seed trays filled with peat on September 5th and transplanted in soil 35 days after sowing (October 10th). Cultivation was carried out in open field conditions at the experimental farm of the University of Thessaly. Four fertilizer treatments were applied: 1) Control (C), 2) farm yard manure (M), 3) standard fertilizer (F; NPK: 20-20-20), and 4) zeolite + standard fertilizer (F+Z; NPK: 20-20-20+Zeolite). For all the fertilizer treatments (M, F and F+Z) the total nitrogen rate was 250 kg ha^{-1}, whereas for F and F+Z treatment nitrogen was applied with three doses (the first dose before transplantation as base dressing and the second and third dose 30 and 60 days after transplantation respectively). In treatments F and F+Z, apart from nitorgen equal amounts of phosphorus and potassium were added (250 kg ha^{-1}), where for M treatment the total amount of added farm yard manure was calculated according to target nitrogen rate (250 kg ha^{-1}). The soil texture was SCL (48% sand, 29% silt and 23% clay). The pH of soil was between 7.7- 8.1 and the organic matter was 1.44%, EC=2.47 dS m^{-1}, CaCO3=2.80% (total) and P Olsen=122 mg Kg^{-1}.
Harvest took place when heads reached marketable size starting 120 days after transplantation. After harvest plant features regarding plant development, such as the total plant fresh weight, the number of leaves, the fresh and dry weight of leaves, heads and shoots were recorded. Fresh samples of leaves were oven-dried at 72 °C to constant weight. Subsequently, the dry samples were powdered using a ball mill, passed through a 40 mesh sieve, subjected to dry ashing in a muffle furnace at 550 °C for 5 h, and used to extract K, Na, Ca, Fe, Mn, Zn and Mg by means of 1 N HCl. The concentrations Fe and Zn in the aqueous extracts were determined by atomic absorption spectrophotometry (Perkin Elmer 1100B) and K by flame photometry (Sherwood Model 410). Phosphorus was extracted with Olsen method according to Sims (2000).
The statistical design was a randomized complete block with four replications per treatment (n=4; 16 plots in total). Each plot was 9 m^2 (3 x 3 m) and plant distances were 50 cm between rows and 40 cm within each row (50.000 plants ha^{-1}). Statistical analysis was carried out with statistical package Statgraphics Centurion (Statpoint Technologies Inc., USA).

Results and Discussion

From the results of the present study it is apparent that fertilizer application, either alone or combined with zeolite (F and F+Z), resulted in significantly higher total plant weight, leaf number and leaf fresh weight, whereas dry matter was higher when no fertilizers were applied

(Table 1). Similar effects were observed for total yield as expressed by head weight, with both fertilizer treatments being higher than farm yard manure (M) and control treatment (C), without however having significant differences with each other (Table 2). Moreover, shoot fresh weight was also higher for fertilizer treatments, whereas dry matter content of head and shoots was higher when farm yard manure was applied (Table 2). Similar results were reported from Bybordi et al. (2013) who concluded that zeolite application can increase plant growth and seed production due to better nitrogen use efficiency and soil amelioration. However, in our study no significant differences were observed between the zeolite+fertilizer and the fertilizer application. These results may be attributed to the fact that plant requirements for nitrogen were fully covered in both cases, therefore the potential of beneficial effects of zeolite were not profound.

Table 1. The effect of fertlizer treatment on plant growth (total plant weight and leaves).

Treatment	Total plant weight (g)	Number of leaves	Fresh weight of leaves (g)	Dry matter content of leaves (%)
C	745.9^{c}	17.6^{b}	290.1^{c}	9.0^{a}
Fertlizer+ Zeolite (F+Z)	1859.7^{a}	20.8^{a}	822.5^{a}	8.0^{c}
Fertilizer (F)	1715.8^{a}	21.1^{a}	773.2^{a}	8.0^{c}
Manure (M)	975.0^{b}	17.8^{b}	396.7^{b}	8.4^{b}
LSD	171.9	0.56	93.5	0.33

*Different latin letters represent significant differences between means of the same column according to Least Significant Differences test (LSD) at p=0.05.

Table 2. The effect of fertlizer treatment on plant growth (heads and shoots)

Treatment	Head fresh weight (g)	Dry matter content of heads (5)	Fresh weight of shoots (g)	Dry matter content of shoots (%)
C	395.4^{c}	7.0^{b}	52.2^{b}	8.2^{b}
Fertlizer+ Zeolite (F+Z)	925.9^{a}	6.0^{b}	103.6^{a}	6.8c
Fertilizer (F)	858.6^{a}	6.2^{b}	97.7^{a}	7.2^{c}
Manure (M)	541.8^{b}	10.3^{a}	56.5^{b}	9.4^{a}
LSD	99.8	1.33	9.9	0.44

*Different latin letters represent significant differences between means of the same column according to Least Significant Differences test (LSD) at p=0.05.

Regarding mineral composition, potassium content in heads and leaves, as well as Fe content in heads, phosphorus content in plant tissues and K and KCl content in soil were not affected by the fertilizer treatments (Tables 3-5). Fe content in leaves was higher for fertilizer treatment when combined with zeolite (F+Z), without however being significantly different from the fertilizer treatment (F; Table 3). Nitrogen content in plant tissues and Zn content in leaves was higher for both fertilizer treatments (F and F+Z), whereas Zn content in heads was higher for F+Z treatment, without significant differences between control (C) and fertilizer (F) (Table 4). Manure application resulted in the highest content of Zn and Fe in soil, without significant differences between C and F in the case of Zn, and F+Z and F in the case of Fe (Table 5). Bernardi et al. (2011) have also reported that zeolite and urea application not only resulted in an increase of dry matter content but also affected mineral composition of silage corn, especially regarding nitrogen content.

Table 3. Mineral composition of heads (K, Fe and Zn) and plant tissues (N) in relation to fertilizer treatment.

Treatment	K (%)	Fe (%)	Zn (%)	Total N
C	3.53	0.009	0.0039^{ab}	0.013^{b}
Fertlizer+ Zeolite (F+Z)	3.84	0.010	0.0042^{a}	0.022^{a}
Fertilizer (F)	3.27	0.009	0.0039^{ab}	0.021^{a}
Manure (M)	3.26	0.008	0.0033^{b}	0.013^{b}
LSD	0.83	0.003	0.0008	0.008

*Different latin letters represent significant differences between means of the same column according to Least Significant Differences test (LSD) at p=0.05.

Table 4. Mineral composition of plant tissues (P) and leaves (Zn, Fe and K) in relation to ferilizer treatment.

Treatment	P (%)	Zn (%)	Fe (%)	K (%)
C	0.073	0.0032^{b}	0.010^{b}	1.96
Fertlizer+ Zeolite (F+Z)	0.079	0.0043^{a}	0.020^{a}	2.51
Fertilizer (F)	0.091	0.0041^{a}	0.015^{ab}	2.29
Manure (M)	0.072	0.0028^{b}	0.011^{b}	2.06
LSD	0.03	0.0008	0.006	0.95

*Different latin letters represent significant differences between means of the same column according to Least Significant Differences test (LSD) at p=0.05.

Table 5. Mineral content in soil expressed in ppm.

Treatmnet	Zn (ppm)	Fe (ppm)	KCl (ppm)	K (ppm)
C	0.52^{ab}	4.28^{b}	698.5	366.7
Fertlizer+ Zeolite (F+Z)	0.47^{b}	5.63^{a}	824.5	349.5
Fertilizer (F)	0.50^{ab}	5.20^{ab}	796.3	417.6
Manure (M)	0.58^{a}	5.65^{a}	754.9	402.7
LSD	0.086	1.30	209.8	79.2

*Different latin letters represent significant differences between means of the same column according to Least Significant Differences test (LSD) at p=0.05.

Conclusion

In conclusion, fertilizer application was very important in order to obtain high yields, since plants had a better growth and finally formed larger heads comparing to the other treatments. Moreover, the application of zeolite had no additional beneficial effect comparing to fertilizer application, however we have to consider its beneficial effects as soil amendment.

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Original scientific paper
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DESIGN OPTIMIZATION OF BOOM SPRAYER BY CFD ANALYSIS

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Abstract

The design optimization of the tractor operated boom sprayer by Computational Fluid Dynamics (CFD)-Analysis has been successfully done for solving a problem of uniform nozzle pressure, spraying fluid losses and energy losses. The new conceptual model of boom sprayer with necessary modifications in old design has been software tested and fabricated for the field performance testing. The performance obtained by modified boom sprayer was stable in all directions after simulation and design optimization in software environment. The uniform length of boom-hosing, nozzle spacing and their orientation was uniform along the boom when measured stationery on a level surface, the distance between the lower edges of the nozzles and the surface was not varying more than 10 cm. The fluid distribution under a developed spray boom was found to be significantly improved when evaluated under the spray scanner. The discharge of **50** lit/min for rated rpm 950 and was able to maintain sufficient pressure in the nozzles. The fabrication of new conceptual design after CFD analysis has been proved successfully for the uniform pressure as well as the 150ml sprayer fluid which is lost during sprayer operations was saved up to 130-140ml.

Keyword: Boom Sprayer, CFD analysis, design optimization, nozzle pressure, simulation

Introduction

Sprayer is an equipment used for application of herbicide, pesticide, and fertilizer to agricultural crops. Tractor operated hydraulic boom sprayer is the most common method of applying pesticides to the field crops in the more developed and relatively wealthy agricultural regions of the world. These type of sprayers have high uniformity of spray, high working efficiency, and full utilization of tractor during idle time. In India, very few research studies have been done on boom sprayers for annual field crops. The existing research studies have showed that variation in nozzle pressure of boom sprayer affect the uniformity of the spray.

The design optimization of tractor operated boom sprayer in computational virtual environment by CFD method is the most useful tool for studying and designing much effective flow control system for spraying equipment to reduce problems arising due to pressure variation in the spray nozzles, which is quite difficult and time consuming with traditional design methods.

Objectives of this research are.

1. Study of existing design and its operational problems of tractor operated boom sprayer.
2. To prepare Computer Aided Engineering Analysis (CAE) report in CAD/CAE-Software.
3. The final confirmation of the boom sprayer specification and fabrication.
4. Field Performance evaluation of modified Boom Sprayer.

Materials and methods

The study of the operational functionality of the existing boom sprayer, modification made in the flow control system, CFD modeling of the flow control assembly of both existing and modified tractor mounted boom sprayer. Experimental set up used for laboratory testing of existing and modified boom sprayer, method adapted for evaluation of sprayer performance.

Existing (old) Boom Sprayer

The pump is driven by the PTO shaft of the tractor and the sprayer unit sucks the chemical and discharges it through the discharge line consisting of a delivery hose to the individual nozzles along the boom. The boom has a swivel arm to direct the spray correctly. The main frame allows the spray boom to be adjusted according to the height of the crops being sprayed. On the frame over which chemical tank and control panel were assembled. The specification of selected sprayer is discussed below.

The design characteristics of various components of the selected boom sprayer are described by constraining the following elements:

Chemical tank b) Main frame c) Boom stand d) Bottom frame e) Boom f) Boom folding arrangement g) Power transmission h) Pump i) Flow Control Assembly j) Spray Nozzles k) Hose pipes

Existing Boom Sprayer (Old):

The control panel consisted of four on-off valves, pressure regulator (PRV) and suck-back valve. It facilitate in spraying from any section of the boom, operating the hydraulic agitator and for suck back valve. Three on/off valves are provided for delivery and one was provided for operating agitator. Pressure regulator was provided for regulating the pressure. Pressure vessel was also provided to reduce the fluctuation of spray liquid is shown in Fig2.1 (a). The control assembly was mounted on the main frame from front side of the sprayer.

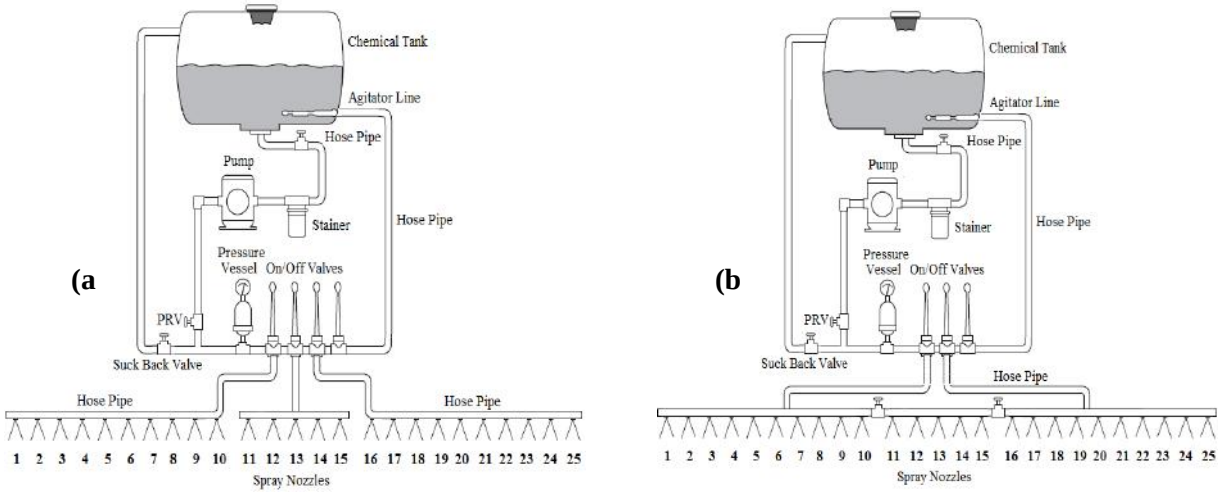

Fig. 2.1 Flow Control System of a) Old Boom Sprayer and b) New Boom Sprayer

Modifications Made in the Boom Sprayer(New):

The modifications in the existing boom sprayer were made only in the flow control system to reduce the variation in nozzle pressure to improve the sprayer performance. The control panel consists of three on-off valves, pressure regulator (CVA) and suck-back valve. Out of These three on-off valves two on-off are connected to the delivery line in such way that the distribution of the nozzles on both sides will be same. The remaining valve operates the hydraulic agitator. Pressure regulator valve is provided for regulating the pressure. Pressure vessel was also provided to reduce the fluctuation of spray liquid is shown in Fig. 2.1(b).

Initial Loading and Boundary conditions for Simulation cycle:
In order to obtain a well-posed system of equations, reasonable boundary conditions for the computational domain have to be implemented. Inlet boundary condition is a uniform liquid pressure at the inlet and outlet boundary condition is the pressure boundary condition, which is set as 101.325 kPa. Wall boundary conditions are no-slip boundary conditions for the liquid phase and free slip boundary conditions for the solid phase and the gas phase. The restitution coefficient for solid – solid has been taken default value of 0.9. The higher viscous effect and higher velocity gradient near the wall have been dealt with the standard wall function method. Table 2.1 shows the boundary and initial conditions for flow control system simulation cycle.

Table 2.1.CFD-Inputs a) Initial conditions

Analysis Type	Internal
Exclude Cavities without Flow Conditions	Yes
Physical Features	
Heat Conduction in Solids	No
Radiation	No
Time-Dependent	No
Gravity	-9.81 m/s^2 in Y Plane
Rotation	No
Fluid Type	Liquid (Water)
Flow Characteristics	
Flow Type	Laminar and Turbulent
Humidity	No
Wall Conditions	
Wall Thermal Condition	Adiabatic
Roughness	0.5 micrometer
Initial Conditions	
Pressure	101.325 kPa
Pressure Potential	Yes
Temperature	303.2 K
Velocity in X-Direction	0 m/s
Velocity in Y-Direction	0 m/s
Velocity in Z-Direction	0 m/s
Turbulence Intensity	2%
Turbulence Length	0.001 m

b) Boundary Conditions

Inlet (for inflow)	
Type	Total Pressure
Flow parameters	Flow vectors direction: Normal to face Total Pressure: 689.5 kPa
Thermodynamic parameters	Temperature: 303.20 K
Turbulence parameters	Intensity: 2.00 % Length: 0.001 m
Boundary layer parameters	Boundary layer type: Turbulent
Outlet (for outflow)	
Type	Environment Pressure
Thermodynamic parameters	Environment pressure: 101.325 kPa Temperature: 303.20 K
Turbulence parameters	Turbulence intensity and length Intensity: 2.00 % Length: 0.001 m
Boundary layer parameters	Boundary layer type: Turbulent

CFD Results of the old and new boom

Sprayer:
The simulated results obtained for the existing boom sprayer have been presented in this section. The pressure and discharge readings predicted by SolidWorks Flow Simulation are presented in Fig3.1 and table 3.1

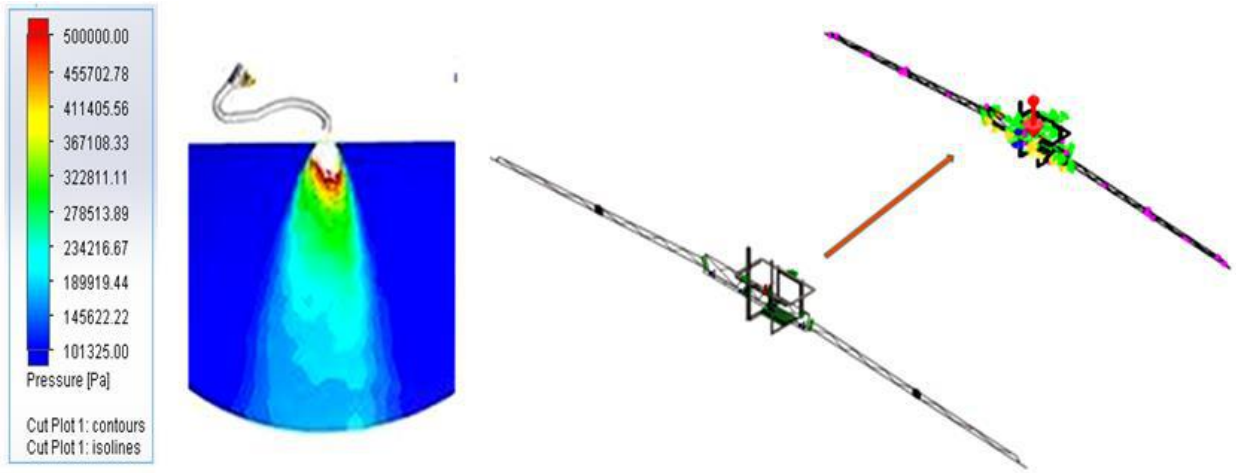

Fig.3.1.Computer Aided Engineering (CAE) Analysis by CFD simulation in boom structure

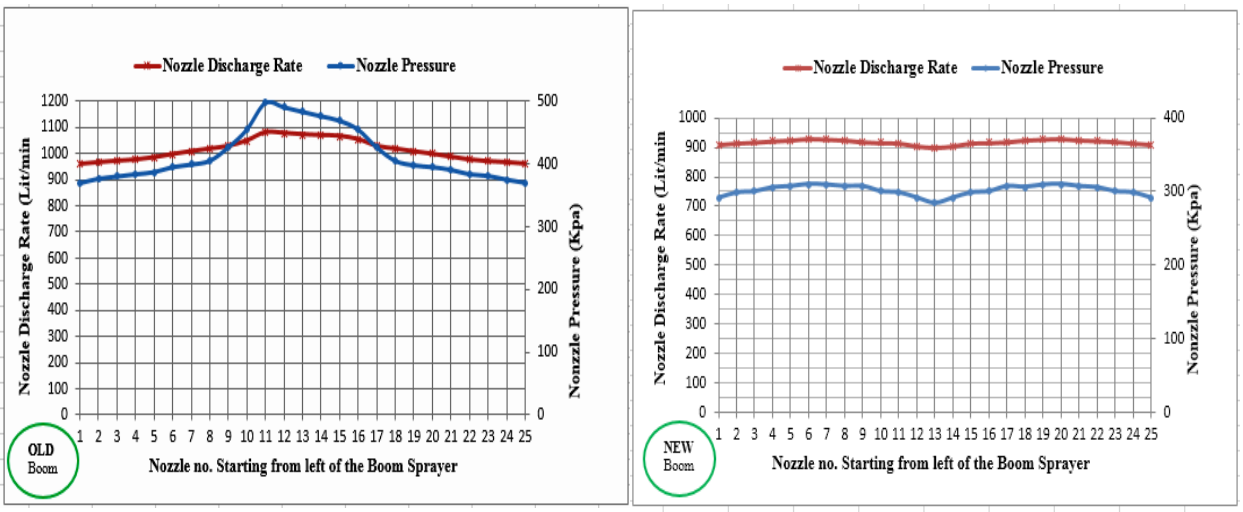

Fig.3.2. Nozzle discharge rate (Lit./Min.) Vs. Pressure (Kpa) for old and new boom design.

Fig.3.3. Nozzle discharge rate (Lit./Min.), Pressure (Kpa) for old and new boom design in Lab.

Table No.3.1: Comparative study of the CFD results and Lab. test results

Nozzles From Left	Old Boom Sprayer (Existing)					
	Pressure (kPa)			Discharge Rate (ml/min)		
	CFD	Lab	ΔP	CFD	Lab	ΔD
1	369	360	9	960	930	30
2	376	370	6	966	940	26
3	380	370	10	972	950	22
4	384	375	9	977	950	27
5	387	380	7	985	960	25
6	395	380	15	997	970	27
7	399	390	9	1008	980	28
8	405	390	15	1018	990	28
9	426	410	16	1028	1000	28
10	455	440	15	1049	1020	29
11	497	480	17	1080	1050	30
12	490	480	10	1077	1040	37
13	483	470	13	1072	1040	32
14	476	460	16	1069	1040	29
15	469	450	19	1066	1040	26
16	455	440	15	1054	1025	29
17	426	410	16	1028	1000	28
18	405	390	15	1018	990	28
19	398	385	13	1007	980	27
20	395	380	15	998	970	28
21	391	380	11	987	960	27
22	384	370	14	977	950	27
23	38	370	11	970	950	20

Nozzles From Left	New Old Boom Sprayer(Modified)					
	Pressure (kPa)			Discharge Rate (ml/min)		
	CFD	Lab	ΔP	CFD	Lab	ΔD
1	291	280	11	910	880	30
2	298	290	9	915	890	25
3	300	290	10	918	890	28
4	305	295	10	922	900	22
5	307	300	7	925	900	25
6	310	305	5	930	900	30
7	309	305	4	928	900	28
8	307	300	7	925	900	25
9	307	300	7	919	900	19
10	300	290	10	916	890	26
11	298	290	9	915	890	25
12	291	285	6	905	880	25
13	284	280	4	900	880	20
14	291	285	6	905	880	25
15	298	290	9	915	890	25
16	300	290	10	916	890	26
17	307	300	7	919	900	19
18	306	300	6	925	900	25
19	309	305	4	928	900	28
20	310	305	5	930	900	30
21	307	300	7	925	900	25
22	305	300	5	923	900	23
23	300	290	10	920	890	30
24	298	290	9	915	890	25
25	291	280	11	910	880	30
Mean			**7.606**			**25.550**
Std. Dev			**2.312**			**3.205**
CV (%)			**30.4**			**12.5**

	1					
24	375	370	5	966	940	26
25	370	360	10	961	930	31
Mean			**12.406**			**27.766**
Std.De.			**3.576**			**3.205**
CV (%)			**28.8**			**11.2**

Conclusions

The pressure and discharge variation of the each individual nozzle was significantly decreased and was able to maintain uniform pressure and discharge in each section of boom.

The new flow control system was able to maintain recommended nozzle pressure of 280 – 300 Kpa at the standard operating pressure of 689.5 Kpa for boom sprayer.

The results predicted by the CFD software simulation environment were slightly higher than results observed during physical laboratory test. But the trend of variation in nozzle discharge rate and pressure was found to be same in both tests.

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Review paper
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COMPARISON OF METHODS USED FOR DETECTION AND IDENTIFICATION OF SOIL-BORNE PLANT PATHOGENS AFFECTING VEGETABLE CROPS

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Abstract

Pathogens that survive in/on the soil and cause diseases in vegetable crops are defined as soil-borne plant pathogens. Soil-borne plant pathogens have always had impact on productivity and they can significantly reduce yield and quality of production. These pathogens are difficult to control because they can survive for long time in the absence of the host crops and often have a wide range of hosts. Detection and accurate identification of soil-borne plant pathogens is one of the most important strategies for controlling soil-borne vegetable diseases. The failure of conventional and culture based morphological techniques for identification and detection of these pathogens has led to the development of serological and molecular detection approaches. The aim of this study is to compare different detection techniques which are being used to detect and identify these pathogens. This paper highlights the brief outlines of traditional detection methods and discusses recent PCR-based techniques being used in detection of these pathogens. The results will show the comparison of methods that have been studied, in order to recommend rapid, sensitive and accurate methods for detection and identification of several soil-borne pathogens affecting vegetables. Therefore, this study is increasingly important for managing and control of soil-borne vegetable diseases.

Key words: *soil-borne pathogens, comparison, detection techniques, PCR, vegetable crops*

Introduction

Vegetable crops represent an important economic segment of the agricultural production worldwide. In 2011, world vegetable production was more than 1 billion tones (FAO, 2013). The production of vegetables, their availability and supply continue to play a major role for the global population. They are important food and highly beneficial for the maintenance of health and prevention from human diseases (Hanif *et al.*, 2006)**.** Although vegetables are susceptible to multiple plant pathogens, soil-borne pathogens present a serious threat in vegetable production. These pathogens are difficult to control because they can survive for long time in the absence of the host crops. The most familiar soil-borne diseases caused by fungal pathogens include *Phytophthora*, *Pythium*, *Sclerotinia*, *Fusarium*, *Rhizoctonia*, *Verticillium.* Among soil-borne pathogens, few diseases are caused by bacterial pathogens, including *Erwinia*, *Rhizomonas*, *Streptomyces*, *Pseudomonas* and *Xanthomonas*. There are very few soil-borne viruses that affect vegetable crops. Viruses generally survive only in the living tissues of the host plants. Indeed, many diseases caused by plant pathogens are difficult to predict, detect and diagnose. In this context, soil creates numerous of barriers to the isolation, detection and identification of soil-borne plant pathogens (Koike *et al.*, 2003). Control of diseases caused by such pathogens usually requires accurate detection, followed by proper identification of the causal organisms. Therefore, preventive measures to avoid planting in contaminated soil are of the highest importance in the context of an integrated approach to control such diseases (Martin *et al.*, 2000; López *et al.*, 2003). As many soil-borne pathogens remain for a long time in the soil, advanced methods of high sensitivity, specificity and reliability are required.

Conventional methods

In the past, conventional methods often rely on identification of disease symptoms, direct isolation in artificial media and laboratory identification by morphology or biochemical tests. In order to perform such techniques was required an experienced and skilled laboratory staff, although, these techniques could lead to problems in identification, incorrect results interpretation, incorrect disease diagnosis and ultimately disease treatments (Atkins and Clark, 2004; Martinelli *et al.*, 2014). In addition, these techniques were time consuming, non-quantitative, prone to contamination or errors and often delay in disease treatments. While conventional techniques are still widely used and are the mainstay of plant pathologists, although molecular approaches are becoming widely available. Therefore, in this review we will focus on the discussion of new molecular techniques which are becoming widely used for the detection and identification of soil-borne pathogens.

Advanced methods

Over the last 30 years, newer techniques have been developed which are being applied in plant pathogen diagnosis. These techniques include: immunological methods, DNA/RNA probe technology and polymerase chain reaction (PCR) (McCartney *et al.*, 2003). Immunological techniques rely on recognition by antibodies of specific antigens either present on the surface of or secreted by the pathogen (Dewey *et al.*, 1997). Immunological methods are available for more rapid presumptive identification, including enzyme-linked immunosorbent assays (ELISA), immunofluorescence colony staining and immuno-strip tests (Hampton *et al.*, 1990; Postnikova *et al.*, 2008). In the recent years, much effort has been devoted to the development of methods for detecting and identifying plant pathogens based on DNA/RNA probe technology and PCR amplification of nucleic acid sequences (Duncan and Torrance, 1992; Martin *et al.*, 2000). The introduction of serological and molecular techniques had a profound impact on plant disease detection and identification.

Results and Discussions

This paper highlights the brief outlines of conventional detection methods and discusses recent molecular techniques which are being used in detection of soil-borne plant pathogens. Table 1 compares detection methods for soil-borne plant pathogens based on their limit of detection, advantages and limitations.

PCR was initially used for highly specific detection of diseases caused by bacteria and viruses (Cai *et al.*, 2014). Moreover, it has been widely used for the detection of soil-borne plant pathogens as well. It is known that PCR depends on the efficacy of DNA extraction and the performance is affected by inhibitors present in the sample assay (Van der Wolf *et al.*, 2001).

Table 1. Comparison of methods for the detection and identification of soil-borne plant pathogens

Techniques	Limit of Detection (CFU/mL)	Advantages	Limitations
PCR	10^3–10^4	High sensitivity, common technology, portable and easy to operate.	Effectiveness is subjected to DNA extraction, inhibitors, polymerase activity and concentration of PCR buffer.
ELISA	10^5–10^6	Low cost, provide quantification and distinguish pathogens taxonomically.	Cross reactions, sometimes lack of specificity and sensitivity.
Conventional techniques	10^7–10^8	Low cost of analysis but sensitivity is not high.	Skilled laboratory staff, low sensitivity, incorrect results, and time consuming.

In addition to the basic PCR technology, advanced PCR methods such as reverse-transcription PCR (RT-PCR) has also been used for plant pathogen identification due to its high sensitivity (López *et al.*, 2003). The greatest advantage of these techniques comparing with conventional diagnostic techniques is the potential to be highly specific. They can distinguish between different plant pathogen species and within a single species (Ward and Adams, 1998). In comparison with conventional methods, molecular methods are relatively fast; results are often possible within one or two days of sampling. They are potentially more reliable than identification of visual symptoms, as they do not rely on the skills needed to distinguish subtle differences in disease symptoms. These techniques have several potential advantages over conventional diagnostic methods in that they are more accurate, they are faster and can be used by personnel with little experience in plant pathology.

Enzyme-linked immunosorbent assay (ELISA) is a popular screening method for identification of diseases based on antibodies and color change in the assay (Fox, 1990). In this method, the target antigens from the plant pathogens are made to specifically bind with antibodies conjugated to an enzyme. The detection can be visualized based on color changes resulting from the interaction between the substrate and the immobilized enzyme (López *et al.*, 2003). ELISA not only gives proof of the presence of a pathogen but is also capable of providing quantification (Cahill, 1999). An advantage of ELISA is that pathogens or a mixture of potential pathogens that may be difficult to distinguish taxonomically can be easily identified using these techniques. The performance of ELISA can be improved greatly with the application of specific monoclonal and recombinant antibodies which are commercially available (López *et al.*, 2001).

Conclusions

In this article we reviewed the conventional techniques and currently existing methods for detection and identification of soil-borne diseases caused by pathogens such as bacteria, viruses and fungi. Based in our study, conventional methods require an experienced and skilled laboratory staff, although these techniques could lead to problems in pathogen identification and incorrect results interpretation. Moreover, these techniques are time consuming, non-quantitative, prone to contamination or errors and often delay in disease treatments. In this review our aim was to compare conventional techniques with advanced methods of pathogen detection. The greatest advantage of molecular techniques comparing with conventional diagnostic methods is the potential to be highly specific. They can

distinguish between different plant pathogen species and within a single species. Molecular techniques are relatively fast, can be performed by personnel with little experience in plant pathology and results are often possible within one or two days of sampling. Although molecular techniques have been used in developed counties, up to date in developing countries such as Kosovo, their application in diagnostic labs has been limited. We do believe that this is about to change in the upcoming years.

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Review paper
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POTENTIAL PREDATORS AND PARASITOIDS REGULATING INSECT PESTS OF MAJOR VEGETABLE AND FIELD CROPS IN SUDAN

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Abstract

Natural enemies are one of the main biotic factors restrained unlimited population buildup of agricultural pests under field conditions. They include predators, parasites and entomopathogens, which normally contribute to natural control that keeps equilibrium of pests below the economic injury level. Hence, exploitation of these enemies in biocontrol through various ways constitutes one of the major components of integrated pest management worldwide. This is important in alleviating the burden imposed by irrational applications of synthetic pesticides on the environment. The Sudan which encompasses various ecological situations is considered rich in its natural flora and fauna. Large numbers of bio-agents were reported to play important roles in natural control of agricultural pests, particularly in areas where slight or no pesticides are used. This paper highlights the potential predators and parasitoids regulating important insect pests of vegetable and field (legumes, cereals, forage) crops in the country. More than fifty predator and forty parasitoid species were listed. The key promising species were suggested to be stressed in future studies for better exploitation in biological control programs, which support the recent trends towards organic farming.
Keywords: *Biological control, natural control, natural enemies, Sudan.*

Introduction

Natural enemies (predators, parasites & entomopathogens) are one of the main biotic factors restrained unlimited population buildup of agricultural pests (viz. natural pest control). Importation, augmentation or conservation of these enemies (biocontrol) comprises an important component of many Integrated Pest Management (IPM) programs globally. But, with the advent of synthetic insecticides during the last century, the trend of pest control in the world has dramatically changed from natural approaches to adopt chemical approach as the sole measure of control. The application of chemicals was started earlier in Sudan where the DDT was used since 1940s. Thenceforth, different organochlorine, organophosphate, organocarbamate and pyrethroid insecticides were used at different periods for combating various pests. Regrettably, the extensive usage of synthetic pesticides has affected the natural enemies negatively (James, 2003; Williams and Price, 2004; Herrera, 1986a). As a result, scientists resorted again to pesticidal alternatives, among which natural enemies were stressed as potential tools in ecologically sound IPM programs.

The Sudan is chiefly an agricultural country which grows several crops for local consumption and export. Sorghum, millet, maize and wheat are the main cereals grown besides leguminous crops, while alfalfa (Lucerne) and Sorghum (Abu-Sabeen) are the important forage grown. Ordinary vegetable crops (e.g. okra, tomato, potato, eggplant, cucurbits, onion, etc) are cultivated in everywhere. However, before the era of pesticide application natural enemies were the major suppressing factors for most agricultural pests. In the Gezira scheme, central Sudan, for instance, more than 200 species of insect and spider predators were said to be found on cotton alone before 1940s (the time when chemical control was started), but less than quarter of that number was hardly detected at the end of the 20th century.

However, the adoption of an IPM program on cotton during the last century proved that the natural enemies prevailing in the Gezira area are capable of controlling *Aphis gossypii* and *Bemisia tabaci* to reasonable levels, provided that broad spectrum chemicals are applied judiciously (Abdelrahman and Munir, 1989). Therefore, great interest was arising to study these enemies and to evaluate their real contribution in pests' control. The surveys and studies conducted yet revealed valuable results (Bashir, 1968; Abu Zinid, 1982; El Abjar, 1985; Satti *et al*., 1998; Satti and Nasr, 2004 and Satti and Bilal, 2012).
This paper aimed to review the previous research findings regarding the major predators and parasitoids associated with the prevailing insect pests of vegetable and field crops in Sudan.

General situation overview

Among polyphagous common insect pests attacking most vegetable and field crops in Sudan are; cotton whitefly (*Bemisia tabaci*), cotton aphid (*Aphis gossypii*), cotton jassid (*Jacobiasca lybica*), cotton thrips (*Thrips tabaci*) and the agromyzid serpentine leaf miners (*Liriomyza* spp.). Various aphids such as cowpea aphid (*Aphis craccivora*) and other thrips, *Caliothrips* spp., generally attack leguminous (e.g. alfalfa) and other crops. Lepidopterous borers like *Earias* spp. mainly attack okra crop, and *Helicoverpa armigera* is a pest of tomato and different legumes. The mentioned pest species and some bollworms are important pests of cotton. Moreover, species of fruit flies (e.g., *Dacus* spp.) are major pests of cucurbits, and tingid bugs for eggplant. Among cereal pests, some aphid species and two lepidopterous stem borers, *Chilo partellus* (spotted stem borer) and S*esamia cretia* (pink stem borer), are the major pests of sorghum and maize. Although, *Helicoverpa armigera* (African bollworm) was reported as minor pest, Kranz *et al*. (1977) emphasized its potentiality as important pest.
The above mentioned pests have natural enemies in common, but specific bio-agents for some closely related pests are also found. Although, research studies on natural enemies in Sudan are sporadically performed, the results reported yet are really fascinating. Most studies were performed on cotton as the important economic crop for the country during the last century. Large number of predator and parasitoid (> 200) species were reported to be found in the Gezira cotton before the era of insecticides usage, but such enemies were drastically reduced thereafter as a result of wide scale spraying of broad spectrum chemicals (after the mid of the 20^{th} century). Earlier studies showed 140 species of natural enemies in 26 families, whereas subsequent research confirmed only 30 species in 17 families (Herrera, 1986b). Fortunately, the IPM program adopted during last decades of the past century has proved that the prevalent natural enemies in the Gezira area are capable of suppressing a number of cotton pests, particularly *Bemisia tabaci* and *Aphis gossypii*, at desirable levels if spraying of insecticides is delayed during the first phase of the cotton growing season (Abdelrahman and Munir, 1989). This encourages research interests on natural enemies, where several crops other than cotton were investigated, though more efforts are still needed. However, studies conducted at different parts of the country proved the richness of local habitats with diversified natural enemies, some of which seemed to be of potential importance.

Predatory species

The important predators reported to be associated with insect pests of most vegetable and field crops in the country are shown in Table (1). Such predators were arranged in the table according to their order and family, scientific names and key prey pests in addition to selected references. Most of these predators were reported from central States including Gezira, Sennar, White Nile, Gedarif and Khartoum (Dabrowski, 1997; Satti *et al*., 1998, and Satti and Nasr, 2004). For example, Satti and Nasr (2004) reviewed more than 40 species of predators belonging to 18 families of insects and spiders to be found in Khartoum State. Moreover, recent studies also manifested the occurrence of large number of predators even in the

northern dryer parts, but largely around the Nile River. Since insecticides are very rarely practiced in that region, numerous species of natural enemies were found flourishing on Table (1). The common predators attacking insect pests of vegetable and field crops in Sudan.

Order:Family	**Species scientific name**	**Key prey species**	**Selected References**
Coleoptera:Coccinellidae	*Coccinella undecimpunctata* L.	Aphids, whiteflies, soft pests.	Bashir, 1968
“	*Coccinella novemnotata* Hbst.	“	Kuol, 2003
“	*Hippodamia variegata* (Goeze)	“	Satti *et al*., 1998;
“	*Cheilomenes propinqua vicina* (Mulsant)	“	Bashir, 1968; Satti *et al*., 1998
“	*Cheilomenes sulphurea* (Oliv.)	“	Kuol, 2003
	Scymnus spp. (e.g., *S. levaillanti* & *S. trepidulus*)	“	Ahmed, 1993; Satti *et al*., 1998; Kuol, 2003
“	*Exochomus flavipes* Thunberg	“	Bashir, 1968
“	*Exochomus nigromaculatus* Goeze	“	Ahmed, 1993
“	*Chilocorus distigma*	“	Ahmed, 1993
Coleoptera: Staphyllinidae	*Paederus fuscipes*	Different pests.	Satti and Nasr, 2004
Coleoptera: Carabidae	Unidentified ground beetle	*Aspongopus viduatus*.	Mukhtar and El Wakeel, 2002
Coleoptera: Meloidae	Larvae of *Epicauta aethiops*	Eggs of Orthoptera (locust).	Mukhtar and El-Wakeel, 2002
Neuroptera: Chrysopidae	*Chrysoperla carnea*	Scales, aphids, whiteflies and lepidopterans	Ahmed, 1993; Satti *et al*., 1998
Dictyoptera: Mantidae	e.g., *Mantis religiosa* &*M.vicoriana*	Different pests.	Satti and Bilal, 2012
“	*Empusa capensis*	Different pests.	Ahmed, 1993
“	*Sphodromantis* spp.	Different pests.	Ahmed, 1993
Diptera: Hybotidae	*Crossopalpus aenescens* (Wiedman)	Adults of *Liriomyza* spp.	Salah *et al*., 2004
Diptera: Syrphidae	*Xanthogramma* (*Ischiodon*) spp. [e.g., *X. aegyptius* (Wiedemann)]	Different aphids, thrips, etc.	Bashir, 1968; Satti *et al*., 1998
Hemiptera: Anthocoridae	*Orius* spp. [*O. albidipennis* (Reut.)]	Aphids, whitefly, dura midge *Stenodiplosis sorghicola*, *Helicoverpa* sp.	Bashir, 1968; Abdelrahman, 1986; Fadlelmula, 2014
“	*Piezostethus* sp.	Different pests.	Ahmed, 1993
Hemiptera: Miridae	*Campylomma nicolasi* Reuter and Puton	Immatures of Lepidoptera (e.g., *Helicoverpa* sp.).	Mahla, 2002; Ahmed and Elamin, 1996
“	*Deraeocori martini*	Different pests.	Ahmed, 1993
“	*Deraeocori ostentants*	Different pests.	Ahmed, 1993
“	*Macrolophus* sp.	Immatures of *Tuta absoluta*	Mahmoud, 2013
“	*Nesidiocoris tenuis* (Reuter) “called Nesibug”	Different pests, e.g., *Tuta absoluta*	Mahmoud, 2013
“	*Stirophylus aristidae*	Different pests.	Ahmed, 1993
“	*Zanchius breviceps*	Different pests.	Ahmed, 1993
Hemiptera: Nabidae	*Nabis capsiformis*	Different pests.	Ahmed, 1993
“	*Pachynomus picipes*	Different pests.	Ahmed, 1993
Hemiptera: Reduviidae	*Phonoctonus principalis* Gerst	Different pests.	Kuol, 2003
“	*Phonoctonus lutescens*	Different pests.	Ahmed, 1993
“	*Coranus aegyptius*	Different pests.	Ahmed, 1993
“	*Cosmolestes pictus*	Different pests.	Ahmed, 1993
“	*Ectomocoris pavoninus*	Different pests.	Ahmed, 1993
“	*Pisra basiptera*	Different pests.	Ahmed, 1993
“	*Prostemma falkensteini*	Different pests.	Ahmed, 1993
“	*Rhincoris albopunctatus*	Different pests.	Ahmed, 1993
“	*Rhincoris segmentarius*	Different pests.	Ahmed, 1993
“	*Rhincoris tibialis*	Different pests.	Ahmed, 1993
Hemiptera: Lygaeidae	*Geocoris* spp. (*G. acuticeps* Signoret & *G. pubescens* (Jak.)	e.g., immature *Helicoverpa*, whitefly, thrips, etc.	Ahmed and Elamin, 1996; Linnavuori,2011

Hemiptera:Pentatomidae	*Dorycoris pavoninus*	Different pests.	Ahmed, 1993
“	*Glypsus conspicuous*	Different pests.	Ahmed, 1993
“	*Macrorhaphis acuta*	Different pests.	Ahmed, 1993
“	*Macrorhaphis spurcata*	Different pests.	Ahmed, 1993
Hemiptera: Berytidae	*Metacanthus mollis*	Different pests.	Ahmed, 1993
Hemiptera: Pyrrhocoridae	*Cenaeus* sp.	Different pests.	Ahmed, 1993
Araneida:	Web weavers, salticids & thomisids	Worms, whitefly, midge, etc.	Satti *et al*., 1998

different crops. For instance, at El-Gorair scheme in Merowe Locality, Northern State, a total of 14 predatory species in 7 families were merely recorded from okra fields (Bilal and Satti, 2012). In such area, some crops like lucerne and date palms which found all the year round were detected as unique habitats in sustaining various predators, particularly during hot summer months when other crops are scarcely found. However, the importance of the recorded generalized predators in ecological pests' control was emphasized in various fields (Herrera, 1986a; Abdalla and Beije, 1997; Satti *et al*., 1998, and Satti and Bilal, 2012).

The important predators found associated with cereal pests may include; spiders (different species), *Chrysoperla carnea*, coccinellids (mainly *Hippodamia variegata*, *Coccinella undecimpunctata*, *Cydonia vicina* and *Scymnus* spp.) and *Campylomma* spp., the latter bug was detected as potential predator of aphids and eggs of *Helicoverpa armigera* showing its peak between September and December. All the previous predators were also found on cotton, vegetables, weeds (e.g., *Abutilon* spp.) and forage crops like alfalfa (Satti *et al*., 1998; Mahla, 2002, and Satti and Bilal, 2012). However, as explained in table 1, the important predators prevailing in most parts of the country are members of coccinellids, chrysopids, syrphids, mantids and spiders. They attack pests from different orders (e.g., Hemiptera, Thysanoptera and Lepidoptera). Among such listed species, *H. variegata* and *C. carnea* showed their wide distribution and highest abundance all the year round as key bio-agents for various pests, particularly aphids and whiteflies. On the other hand, *C. carnea* and spiders were potent enemies of tingid bugs and lepidopterous insects. This suggests the importance of the two predators in regulating these pests on various crops. These predators should receive great attention to enhance their role in pest management programs.

Important parasitoids

Regarding the parasitoids, table (2) listed the most important species associated with pests of vegetable and field crops. The whitefly seems to be fairly controlled with two parasitoids (viz., *Encarsia lutea* and *Eretmocerus mundus*), especially in areas of low pesticidal usage. These two species were encountered throughout the year on different host plants, hence, they were recommended for further studies to be utilized efficiently in biocontrol. Similarly, a number of larval-larval and larval-pupal parasitoids were reported to attack leaf miners (*Liriomyza* spp.) at variable levels on various crops as well as on different wild plants. Some of these parasitoids were found to exert high levels of parasitism on *Liriomyza* larvae attacking economic vegetable and field crops like cotton, okra, beans, peas, cucurbits, solanaceous crops and others. So, they reflect high potentiality to be tested for the biocontrol of these devastating pests. However, parasitism on leaf miners was found to be higher at Dongola area in northern Sudan (may reach > 81%) than at the Gezira area, which attributed to very limited insecticides usage in the former region (Ibrahim *et al*., 2004).

Parasites reported for *Chilo partellus* are; *Vipio deesae* and *Euvipio* sp. as larval parasites, and *Hyperchalcidia soudanensis*, and *Pediobius* sp. as pupal parasites. On the other hand, the egg parasite, *Platytelenomus hylas*; the larval parasites, *Apanteles ruficrus*, *Apanteles sesamiae* and *Bracon brevicornis*, and the pupal parasites, *Brachymeria sesamiae* and *Pediobius furvus*, were recorded for *Sesamia cretica*. However, nine parasitic insects were recorded for *Helicoverpa armigera* (Schmutterer, 1969; Balla, 1986, and Kranz *et al*., 1997), a major pest

of various crops in the Gezira area. A recent study in Khartoum State showed a number of parasites on the three previous pests, with the highest parasitism (> 62%) being recorded on *H. armigera* by *Apanteles* sp. (Satti, 2007). The latter parasite and spiders' predators were the dominant bioagents in this area. However, in agricultural schemes of central Sudan (e.g., New Halfa) *Diplazon laetatorius* and tachinid flies *Tachina* sp. are the dominant parasitoids on *H. armigera* (Mohamed, 2011). Winter season manifested higher impact of natural enemies on these pests than the other seasons.

Table (2). The important parasitoids attacking most insect pests of vegetable and field crops in Sudan.

Order:Family	**Species scientific name**	**Key prey species**	**Selected References**
Hymenoptera: Aphelinidae	*Aphelinus maidis* Timberlake	*Aphis craccivora* and other pests	Bashir, 1968
“	*Aphelinus sudanensis* El Abjar	*Aphis gossypii*	El Abjar, 1985
“	*Encarsia* spp.(e.g.*E.lutea* Masi)	*Bemisia tabaci*	Abdelrahman, 1986
“	*Eretmocerus mundus* Marcet	“	Satti and Nasr, 2004
“	*Eretmocerus diversiciliatus*Silv.	“	Abdelrahman, 1986
Hymenoptera: Braconidae	*Apanteles disparopsidis* Lyle	Bollworms (e.g., *Helicoverpa armigera*)	vandenBerg *et al*., 1988
“	*Apanteles ruficrus* (Hal.)	*Helicoverpa*, *Spodoptera* & *Sesamia*	Bashir, 1968; Satti, 2007
“	*Apanteles sesamiae* Cam.	Stem borers (e.g., *Sesamia*)	Schmutterer, 1969
“	*Bracon* (=*Microbracon*) *brevicornis* Wesm.	Stem borers (*Sesamia* & *Chilo*) & bollworms (e.g., *Helicoverpa armigera* & *Earias insulana*).	Schmutterer, 1969; Mokhtar and El-Wakeel, 2002; vandenBerg *et al*., 1988
“	*Bracon* (*Habrobracon*) *concolorans* (Marshall);[Syn. *B. nigricans* (Szepligeti)]	Larvae of *Tuta absoluta*	Mahmoud, 2013
“	*Bracon* (*Habrobracon*) *hebetor* Say	Larvae of *Spodoptera exigua*, *Helicoverpa armigera*, …etc.	Bashir, 1968; Ahmed and Elamin,1996;Mahmoud,2013
“	*Bracon kirkpatricki* Wesm.	Bollworms (e.g., *Helicoverpa* sp).	Schmutterer, 1969
“	*Chelonus bifoveolatus* Szepl.	*Spodoptera exigua*	Bashir, 1968
“	*Chelonus curvimaculatus* Cam.	Bollworms like *Helicoverpa*, *Spodoptera* &*Pectinophora*.	Bashir, 1968
“	*Chelonus versatilis* (Wilkinson)	Bollworms (e.g., *Helicoverpa* sp).	Ahmed and Elamin, 1996
“	*Meteorus laphygmarum* Brues	Bollworms (e.g., *Helicoverpa* sp).	Ahmed and Elamin, 1996
“	*Opius dissitus* Muesebeck	Leaf miners (*Liriomyza* spp.)	Ibrahim *et al*., 2004
“	*Stenobracon* (*Vipio*) *deesae* (Cameron)	Larvae and pupae of stem borers e.g., *Chilo partellus*	Nasr El Din, 1965
“	*Trioxys complanatus* Quilis	*Therioaphis trifolii maculata*	El Abjar, and Bashir, 1989
“	*Zelomorpha sudanensis* Gahan	*Spodoptera exigua*	Bashir, 1968
Hymenoptera: Chalcididae	*Brachymeria bottegi*	Different pests	Ahmed, 1993
“	*Brachymeria sesamiae* Gah.	Stem borers (e.g., *Sesamia* spp.), etc	Ahmed, 1993
“	*Hyperchalcidia soudanensis* St.	Pupae of *Chilo* spp. & other borers	Schmutterer, 1969
Hymenoptera: Elasmidae	*Elasmus johnstoni* Ferrière,	Bollwrms (e.g., *Helicoverpa armigera* & *Earias insulana*).	Mokhtar &El-Wakeel, 2002; Ahmed and Elamin, 1996
Hymenoptera: Eulophidae	*Aprostocetus* sp.	Sorghum midge (*Stenodiplosis sorghicola*)	Fadlelmula, 2014
“	*Cirrospilus* sp.	Leaf miners (*Liriomyza* spp.)	Salah *et al*., 2004
“	*Diglyphus isaea* (Walker)	Leaf miners (*Liriomyza* spp.)	Salah *et al*., 2004
“	*Euplectrus laphygmae* Ferriore	Larvae of bollworms (e.g., *Helicoverpa* & *Spodoptera* spp.).	Ahmed and Elamin, 1996; vandenBerg *et al*., 1988
“	*Hemiptarsenus semialbicalva* (Girault)	Leaf miners (*Liriomyza* spp.)	Salah *et al*., 2004
“	*Neochrysocharis*	Leaf miners (*Liriomyza* spp. and	Sharaf Eldin *et al*., 1997;

	(=*Closterocerus*) *formosa* (Westwood)	*Tuta absoluta*)	Mahmoud, 2013
“	*Pediobius furvus* (Gahan) & *Pediobius* sp. (Walker)	Stem borers (e.g., *Sesamia* & *Chilo* spp.); bollworms (e.g., *Heicoverpa* sp.); Leaf miners (*Liriomyza* spp.)	Schmutterer, 1969; Ahmed and Elamin, 1996; Salah *et al*., 2004
“	*Tetrastichus* sp.	Sorghum midge (*Stenodiplosis sorghicola*)	Fadlelmula, 2014
Hymenoptera: Figitidae	*Kleidotoma favus* Quinlan	Leaf miners (*Liriomyza* spp.)	Salah *et al*., 2004
Hymenoptera: Ichneumonidae	*Diplazon laetatorius* (Fabricius)	*Helicoverpa armigera*; but also parasitize syrphidflies	Mohamed, 2011 Bashir, 1968
Hymenoptera: Pteromalidae	*Anisopteromalus calandrae* (Howard)	*Callosobruchus maculatus*	Abu Zinid, 1982
“	*Halticoptera circulus* Walker	Leaf miners (*Liriomyza* spp.)	Ibrahim *et al*., 2004
Hymenoptera: Scelionidae	*Platytelenomus hylas* Nixon	Stem borers (e.g., *Sesamia* sp.)	Schmutterer, 1969
“	*Telenomus busseolae* Gahan	Eggs of *Helicoverpa armigera*	Ahmed and Elamin, 1996
Hymenoptera: Torymidae	*Ecdamua cadenati* (Risbec)	Larvae of *Tuta absoluta*	Mahmoud, 2013
Diptera: Tachinidae	*Carcelia evolans* (Wied.)	Larvae of bollworms (e.g., *Helicoverpa* sp,*Diparopsis watersi*)	Ahmed and Elamin, 1996
“	*Exorista* spp.	Different pests	Ahmed, 1993
“	*Goniophthalmus halli* (Mesni)	Larval-pupal stages, *Helicoverpa* sp.	vandenBerg *et al*., 1988
“	*Palexorista laxa* (Curran) & *Palexorista* sp.	Larvae of *Helicoverpa armigera* & *Spodoptera exigua.*	Bashir, 1968; Ahmed and Elamin, 1996
“	*Strobliomyia palaestina* (Vill.)	*Spodoptera exigua*	Bashir, 1968
“	*Tachina ebneri* Villeneuve, “locally called Um-Sigara “	*Helicoverpa armigera* & *Auchmophila kordofensis* Rebel	Mohamed, 2011 Mahmoud *et al*., 2006

In spite of what have been mentioned regarding those abundant natural enemies, no recent studies are found considering biological control of economic pests in the country. The indigenous natural enemies reported yet for most insects are so encouraging to continue forward in studying their potential role in IPM, particularly those of aphids, whiteflies, African bollworm and cereal pests like stem borers. In contrast, it is clear that some economic pests like jassids (*Jacobiasca lybica*), flea beetles and some other major pests have no promising parasitoids known. Therefore, Kleks and van Lenteren (1991) who reviewed the situation of jassids recommended that the role of local natural enemies in controlling *J. lybica* should be clarified firstly before attempting introduction of any natural enemies to the country. Nevertheless, they suggested *Anagrus atomus* as the most promising parasitoid to be considered for importation. Also, a number of parasitoids listed in the table were reared from different pests, but they lack sufficient investigations to understand their real contribution in natural control. More research is needed to cover the various bio-ecological aspects of such enemies and to evaluate their importance in combating different pests.

Conclusion

Abundant predators and parasitoids of agricultural pests are found in Sudan. Some species showed potential role in combating certain major insect pests like aphids, whiteflies, tingid bugs, leaf miners, bollworms (particularly *Helicoverpa armigera*) and cereal stem borers. Areas of higher pesticide application showed apparent negative impact on the populations of natural enemies. However, most of the recorded species lack sufficient evaluation regarding their potential role in pest control. Hence, the paper stressed the importance of studying these bio-agents for better exploitation in integrated pest management.

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Original scientific paper
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COMPOSITION ANALYSIS OF THE SELECTED FOOD SUPPLEMENTS

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Abstract

Rapid pace of life, exposure to stress and diet based on highly processed foods, make people want to enrich their food by appropriate supplementation. For this reason in many highly developed countries (also in Poland), dietary supplements market is developing very dynamical and it is mostly powered by non-pharmacy sales. There are no specific regulations on the components of dietary supplements other than vitamins and minerals it makes the market for these products is characterized by considerable diversity. We often hear of undervalued quality dietary supplements. Particularly big problem with maintaining quality supplements are extracts from plants. In this study author described the results of own research on composition of supplements containing omega-3 fatty acids and supplements containing extract of Ginkgo biloba. In first group of analyzed products the content of EPA was mostly in line with the declaration of the manufacturer and pharmacological requirements were fulfilled. (quality, safety and effectiveness of use) The fatty acids of the dietary supplements transfer into methyl esters (FAME) and analyzed by using gas chromatography (GC). The analysis was made in triplicate. In case of supplements with extract of Ginkgo biloba, some products had correct level of standard extracts of Ginkgo biloba and had fulfilled pharmacological requirements. But in this group were also products with too low amount of standard extracts and elevated ginkgolic acids (this is potentially toxic substance for human).
Keywords: *food supplement, dietary supplements, omega-3, plant's extracts, Ginkgo biloba*.

Introduction

Dietary supplements market in Poland is developing very dynamically and it can be bought not only in pharmacies, but also in supermarkets, drug stores, what makes go up the sale of these preparations. Last year society in Poland spent on them more than 2.8 billion zł. According to the surveys this amount in upcoming years will grow by 9-10 percent per annum. Dietary supplements are mostly foodstuffs that are intended to supplement our diet with vitamins, minerals or other substances having a nutritional. These preparations can be purchased in the form of capsules, dragees, sachets of powder, tablets or ampoules with liquids and other similar liquid forms and powders designed to be eaten in small, measured unit quantities, excluding products which have the properties of a medicial product within the meaning of pharmaceutical legislation (Dz. U. 2010. No. 136, pos. 914 as amended). (Krasnowska and Sikora, 2011) Chief Sanitary Inspector in Poland is in charge of a supervision of dietary supplements. The introduction of these products on the market requires less research and fewer demands than in the case of drugs. So why so many people are willing to use for these products? All of this is caused by because magic of the advertisements, in which producers have taken care of the supplements to be associated more with medicine, which can easily, cheaply and quickly improve our health. According to the Regulation of the Minister of Health from 2007 supplements may include vitamins or nutrients, but also other additional substances that do not always have a positive effect on our body and are not strictly specified by pharmaceutical law. According to a European Commission report the number of substances used in the manufacture of food supplements is approx. 400 from this amount as

50 percent of the market of these formulations in the European Union are the vitamins and minerals.

Materials and methods

In 2014, as a part of scientific extra classes OrgChem author had performed 7 months analysis of the composition of dietary supplements with contains omega- 3. In research author used not only dietary supplements containing omega- 3 for adults but also for children and pregnant women. Work was performed with 15 different products, which were the main source of fish oil, fish oil and algae. The names of supplements that are used for testing are 1 Möller 's Tran Omega-3 DHA Biovilon 2. 3. Lupicol DHA, 4. Olympus Gold Omega -3, Omega -3 5. mite, 6. Omegamed Pregna DHA, 7. Pregna 250 DHA, 8. Prenatal DHA from algae, 9. Rekinal, 10. Tran, 11. Omega -3 Forte, 12. Ecomer, 13. Olimp Gold Omega 3 Forte, 14. Omega -3 Platinum, 15. Doppel Herz activ mama. The fatty acids of the dietary supplements transfer into methyl esters (FAME) and analyzed by using gas chromatography (GC). The analysis was made in triplicate. The results of the research conducted on Gingo biloba have been taken from the literature.

Results and discussion

Omega-3 and omega-6 are polyunsaturated essential fatty acids (WNNKT). They are not synthesized by the human body and thus must be supplied in the diet. The ratio of omega-3 to omega-6 should be 1:2 or 1:5. Omega-3 acids are: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Shahidi and Wanasundara, 1998). The human body is able to change linolenic acid into EPA and DHA. The first of these acids is rooted in flaxseed, hazelnuts, rapeseed oil and soy. In contrast acids DHA and EPA are mainly found in fish, seafood, as well as algae. The greatest amount of omega-3 is located in salmon (1.2 g / 100j of the product) (Breivik, 2012). In order to ensure proper absorption of polyunsaturated fatty acids ingestion should be accompanied by vitamin E, which is a natural antioxidant (Kolanowski, 2007). Diet has a huge impact on human health. Cardiovascular diseases are the leading cause of death in many countries. Reducing the consumption of saturated fatty acids and an increase in polyunsaturated is an effective way to reduce cholesterol levels in the blood. Consuming omega-3 has anti-atherosclerotic effect, anti-cancer effect, limits the development of diabetes type 2, regulate lipid metabolism and have a positive effect on the condition of skin that becomes more moist and supple. Moreover, DHA is essential for the proper development of the brain and retina (Falinska and Bascoul-Colombo, 2012).

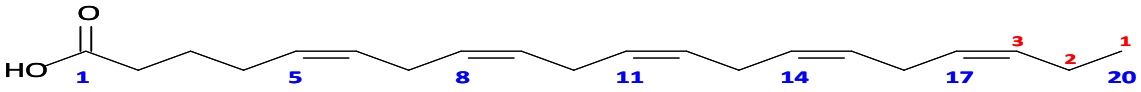
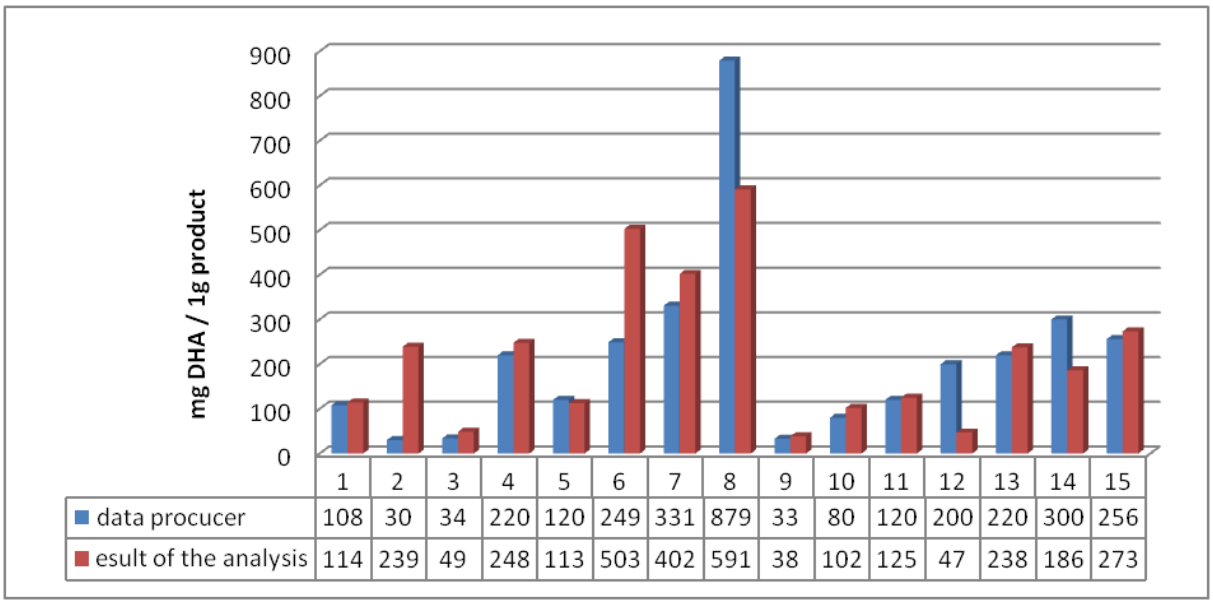


Figure.1. Docosahexaenoic acid.

Figure.2. Eicosapentaenoic acid.

Consumers are increasingly turning to supplements, which may provide them with the right amount of omega- 3 because fish from unknown sources contain increased amounts of heavy metals such as lead and mercury. Also, pregnant women decide to supplement the diet with these acids to prevent retinopathy of premature of in babies and to ensure proper brain development in children (Siddiqui and Zerouga, 2006; Anna Walczewska and Tomasz Stępień, 2011).

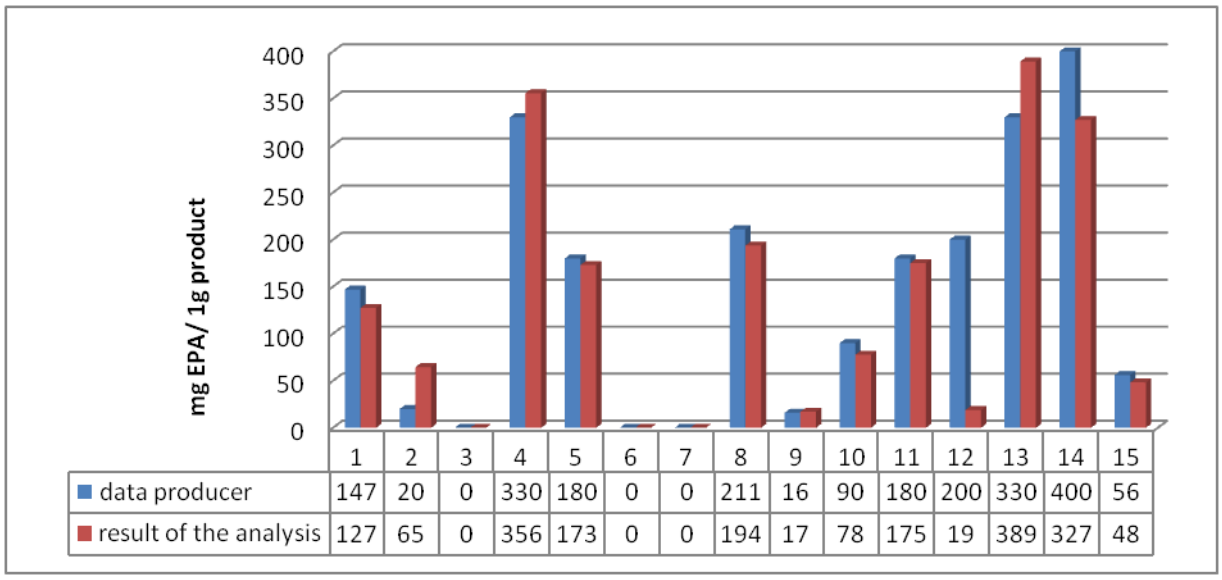
Test results for DHA. The analysis results were quite surprising. None of the supplements contained the declared content of the acid. In most cases, the manufacturer stated the higher amount, so the client received gratis from producer. Only in a few cases the content was lower than the under stated the amount of, we were deceived, and the manufacturer took a given amount of product. Most additional component in the form of DHA is in products: 2. Biovion DHA DHA 6. Omegamed Pregna and 7 Pregna 250 DHA, are products for supplementation of pregnant women and children under 6 years of age. However, in products: 8 Prenatal DHA from algae, Ecomer 12 and 14. Omega-3 Platinum most of the acid was stolen. It may also result from the fact that the sources of these supplements are mainly sea algae containing small amounts of DHA. In other cases, the deviation was within the normal range.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
data procucer	108	30	34	220	120	249	331	879	33	80	120	200	220	300	256
esult of the analysis	114	239	49	248	113	503	402	591	38	102	125	47	238	186	273

Figure 3. Check the manufacturer’s assurances for DHA

The test results for acid EPA. For the EPA acid is the same analysis as for DHA was performed. According to the data given on the packages in three formulations: 3.Lupicol DHA DHA 6.Omegamed Pregna, 7. Pregna 250 DHA EPA does not exist, as confirmed by analysis. These preparations are meant to be for pregnant women and for children under 6 years of age. The biggest surprise was for the preparation 12. Ecomer because analysis showed a trace amount of acid in the preparation. Understated ingredient content was also observed in formulations such as: 1. Möller 's Tran Omega -3, 8 Prenatal DHA from algae and 14. Omega -3 Platinum. The two latter products also contain DHA acid content understated . We may be given some gratises when buying producks: 2. Biovion DHA, 4. Olimp Gold Omega -3 and 13. Olimp Gold Omega 3 Forte .



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
data producer	147	20	0	330	180	0	0	211	16	90	180	200	330	400	56
result of the analysis	127	65	0	356	173	0	0	194	17	78	175	19	389	327	48

Figure 2. Check the manufacturer’s assurances for EPA

Research on nutritional supplements containing Japanese ginkgo biloba were made by scientists from the University of Life Sciences in Poznan. It has been tested as many as 11 of ginkgo biloba preparations. Main active compounds and toxic compounds were determined in these foodstuffs. The research included qualitative analysis and determinated the content of flavonoid compounds, and terpene lactones ginkgolic acids. After hydrolysis, the content of flavonoids formulations was similar to the declarations of manufacturers as well as to the requirements of the pharmacopoeias. Only in four products the content of quercetin and kaempferol (flavonoids) coincide with the data on the packaging, in other supplements the amount of quercetin was higher and the amount of kaempferol was lower, which indicates that the supplements do not contain the full value of ginkgo leaf extract. However, high levels of acids tested ginkolie in 4 dietary supplements (in one case the standard has been exceeded 1,600 times) may result from the use of of non-standardized extracts in production. Increased amount of ginkgolic acid content is considered potentially toxic.

Dietary supplements containing vitamins, minerals, fatty acids, amino acids, probiotics and prebiotics, of course, we can take in moderate doses , in states of increased demand. Instead, we must be careful with products containing vegetable raw materials outside Europe. They contain substances of different from those used traditionally in our latitude. These plants grow in different soils, are used among the population, which otherwise he eats, it has a different metabolism. What for people in Asia or South America is good can be devastating for us. According to the EFSA guidance, supplements containing plant materials previously not used as food or used for other purposes, shall undergo examinations proving the safety of their use. According to the law, for the safety of supplements is the responsibility of the manufacturer. This is a large pharmaceutical company that has implemented GMP system, we can assume that the product is of sufficient quality. But if you do not know anything about the manufacturer, it is such certainty we no longer have.

Conclusion

Dietary supplement, as the name suggests aren’t a medicinal product. It can be helpful in addition to certain missing elements or vitamins in the body, but must be used in moderation and preferably under the supervision of a specialist - a doctor or pharmacist. Given the abundance of available, often contradictory information on dietary supplements, patients need help in getting to reliable sources about dietary supplements and proper nutrition. Analyzing the content of dietary supplements can be assumed that they do not meet the requirements of

the manufacturer. Often the component that is less expensive or more easily extracted is present in greater amounts / concentrations in the product. Given the supplements on the package does not match the actual composition after analysis.

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THREE SUCCESSIVE TIMELY WORK MASKED GENES SAVING OF VIRULIFEROUS INSECTS AND COORDINATED WITH ZYMV RESISTANCE GENE IN SQUASH.

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Abstract

Binary and ternary hybridization among three pure lines previously isolated having different kinds of white silvery colors were done to know how the saving genes of *Zucchini Yellow Mosaic Potyvirus* (ZYMV) viruliferous insect will react with each other's, and to compare some new parameters of different genes included genotype. Characters efficiency (a ratio between the saving period and the character appearance period) measuring revealed that they were 92.8, 94.4, 76.6%, for the full leaf silvery color, netty white, white spotted respectively, and for all characters jointly was 63.3%. The Saving period (summation of the lasting days of the genotype first infection after control and the number of days of full infection dissemination in the genotype unite) of pure lines, binary and ternary hybrids were also estimated. Results were 13.0-20.0, 25.0-29.0, and 39.0 days respectively. The Saving degree (a ratio of the different between the saving period of the genotype and the dissemination period in control to the saving period of the genotype) was 0.46 - 0.65, 0.70 – 0.75, and 0.79 The Saving efficiency (the saving degree multiplied by the character constant) was 46.0 – 65.0, 70.0 – 75.0, and, and 79.0%.There were an interaction and operation sequences of the three characters appearance (the previous covered or masked the post), where the beginning with full leaf silvery color followed by netty white and then white spot. More over in quaternary hybrid that has three of saving genes (characters) and one of ZYMV resistant. Resistance degree (the different between the highest resistance degree (1.0) and the genotype infection degree) was 0.83. The Resistance efficiency (the resistance degree related to 100%) was 83%. Protection efficiency (summation of saving efficiency and resistance efficiency related to 200) was 182/200.

Key words: *Vegetable breeding, plant virus resistance, masked gene, squash leaf coloration.*

Introduction

Zucchini yellow mosaic Potyvirus (ZYMV) causes considerable losses of cucurbitaceous vegetables grown nearly all over the world; indeed, the commonly planted cultivars are highly susceptible to ZYMV (Svoboda et al., 2013). Genetic resistance is the best manner to control viruses especially that of insect transmitting. Aphid transmitted many viruses infected squash (*Cucurbita spp*) (Boyhan, 1999; Davis and Shifriss, 1983; Shiffriss, 1981; Shiffriss, 1982; Smith et al., 1964) causing 100% high severity infection and high yield losses most times. Insecticide controlling of virus victor commonly is not effective manner to reduce virus infections (Boyhan, 1999). Reflective mulches have been used to reduce plant insect visitations and so virus infections (Hrpaz, 1982; Kring, 1964; Kring, 1969; Kring, 1969; Moore et al., 1972; Wyman et al., 1979), but it is expensive, difficult to apply, and mostly be ineffective when plants cover the ground surface or in state of the vector pressure is too high (Davis and Shifriss, 1983). In the same way white silvery leaf coloration of squash have been shown to reflect light and so it alter aphid flight activities and repel them, or reduce the attractiveness of the landing site (Davis and Shifriss, 1983; Shiffriss, 1981; Shiffriss, 1982).

Squash leaf coloration is a compound character appears in several forms for limited periods. They may interact with each other shortly. They have a role of saving squash plants of virus vectors insect. They differs in their density, size or power of expression depending on genotype, environment factors (sun solar intensity, temperature, and relative humidity), or they affect by the insect pressure and injury severity (Davis and Shifriss, 1983; Shiffriss, 1981; Shiffriss, 1982).

In fact the saving or resistance traits be in different levels, but there were not grades express by accurately reflect degrees of resistance or saving to differentiate characters within one genotype or one character among different genotypes. So needing arose to use precise measurements and developed a new mathematical relations to compare different aspects of coloration saving, or resistance to ZYMV in one or different genotypes by giving every coloration shape the degree or ratio of saving or resistance extremely, and to get the real saving or resistance efficiency in each genotype, or to compare resistance strains or hybrids included than that free, or any other breeds or varieties possess some level of resistance or to evaluate the performance of any material against any disease.

Material and Method

Four strains of squash different form coloration, full silver leaf 2020A, netty white 2020B, white spotted 2020C, and control free of coloration (full green leaf) 2020D, also binary, ternary hybrids among these lines, and quaternary with ZYMV resistance strain were planted in rows of 5m length, distance between one and another 2 m, and 0.75 m between the plant and the last, with three replications, in three sites within 5 km between site and another, according to randomized complete block design. Plants in each replicate were left for natural infection from sensitive squash plants that were mechanically infected by ZYMV in transverse lines of 4m long at a distance 5m on top of each replicate as a source of infection by insects of peach green aphid (*Myzus persicea*) that were put and left to proliferate on each plant. These insect were previously tested to their free of any virus by put on susceptible squash plant. To see the indirect impact of the presence of each shape of coloration to delay the initial infection and the naturally dissemination of ZYMV in the field, the period from the appearance of each character of coloration to disappearance were recorded, as well as from begin of the initial infection to complete distribution on plants of each strain in each duplicate. Saving period, Saving degree, Saving efficiency, and Health yield percent for each coloration form in each line separately, and in binary, ternary crosses of these genotypes, more over to correct comparison between the coloration forms in different genotypes, in addition to extract the real values of these parameters it has developed a new mathematical relation as following:

Character constant (Character efficiency) = $\frac{Character\ saving\ period}{Character\ appearance\ period\ mean} \times 100\%$

Control lasting period = $No.of\ days\ to\ first\ infection + No.of\ days\ to\ full\ infection\ dissimination$

Genotype saving period = $No.of\ days\ to\ first\ infection\ after\ control + No.of\ days\ to\ full\ infection\ dissimination$

Genotype saving degree = $\frac{genotype\ saving\ period - control\ lasting\ period}{genotype\ saving\ period}$

Genotype saving efficiency = $saving\ degree \times character\ constant$

Health yield percent = $\frac{Health\ yield}{Total\ yield} \times 100\% = \frac{period\ of\ health\ yield}{period\ of\ total\ yield} \times 100\% = \frac{yield\ savig\ period}{period\ of\ total\ yield} \times 100\%$

As well as in quaternary hybrid of the three coloration form with the resistant strain, disease severity was divided into the following grads of disease scale: 0 = No symptoms of injury, 1= leaf mottle or mosaic, 3= complete yellow mosaic and blisters on leaves and fruits, 4= leaf curl or simple deformation of leaf and fruit, 5= leaves turning into high strings or strong leaves and fruit deformation. Infection degree, resistance degree, resistance efficiency, and prevention efficiency, were calculated by used the following relations:

$$Genotype\ infection\ degree = \frac{\max grade\ of the\ genotype\ infection}{No.of\ grades\ of disease\ scale}$$

$$Genotype\ resistanc\ degree = Absulute\ degree\ of\ resistance - Genotype\ infectio\ degree$$
$$= 1 - \ Genotype\ infectio\ degree$$

Genotype Resistance efficiency $= resistance\ degree \times 100\%$

$$\text{Prevention efficiency } = \frac{saving\ degree \times 100\% \ + resistance\ degree \times 100\%}{200}$$

Results and discussion

The white silvery coloration traits of squash leaves have been separated into three different forms pure line in a previous study (Fig 1). Results of appearance of each form individually or upon entry into different hybrids (Table 1) revealed that these periods were differed from one format to another; as they have ranged between 8.2-41.0 days in pure lines, and the longest visible was spotted white. In hybrids appearing periods were doubled by adding period of any shape to the others interred with it in the hybrid, where in binary hybrids has increased to range between 20.0 - 69.0 days, and the longest period was the hybrid (netty white × spotted white) for collect two coloration forms entrants in it, and have increased more in ternary hybrids to 35.4 - 83.7 days for periods of three forms gathered together. That's mean as the greater the number of formats increased in the genotype the appearance period increased of being appeared in sequence and not in the same time. This represents the max aim of the collection of shapes in hybrids.

Silver full leaf

Netty white

Full silver to netty white

Netty white to spotted white

Full silver to spotted white

Fig (1) Different squash leaf coloration form and their short interference through sequential appearance in hybrids.

But although the appearance periods have increased in these hybrids approximately to the age of plant that of 100 days, the real periods of saving represented by the initial infection lasting and periods of infection dissemination did not exceed in the maximum, but only to the half age of plant or two third of productivity period almost, where the Initial infection lasting periods (Fig 2) has ranged in the pure lines 7.0 -11.0 days and the best was in the hybrid (netty white × spotted white), and more increased in ternary hybrids to 15.0 days, as well as of infection dissemination periods in experimental units ranging in the pure lines of the coloration forms between 7-13 days and the highest being spotted white, and in binary hybrids between 15-18 days and the best was (netty white × spotted white) as well, and arrived in ternary hybrids to 24 days. This has served the saving periods to increase from 13-20 days in the pure lines and 24-29 in the binary hybrids and to 39 days in ternary hybrids.

Table 1. Relation of character, saving, prevention efficiency, and health yield ratio with the appearance and saving period

Genotype coloration	Period of Character appearance (day)	Period of first infection lasting (day)	Period of Disease Dissemination (day)	Saving (lasting) period (day)	Character Constant %	Saving Degree	Saving Efficiency %	Prevention Efficiency /200	Health yield %
No coloration (Control)	0.0	4.0	3.0	7.0	0.0	0.0	0.0	0.0	0.0
Full silver leaf	8.2 – 20.2	6.0	7.0	13.0	92.8	0.46	46.0	46.0	25.0
Netty white	10.0 – 26.0	9.0	8.0	17.0	94.4	0.58	58.0	58.0	28.3
White spotted	11.2 – 41.0	7.0	13.0	20.0	76.6	0.65	65.0	65.0 .	33.0
Binary hybrid: Silver Full leaf ×netty white	20.0 – 49.2	10.0	15.0	25.0	84.1	0.70	70.0	70.0	42.0
Binary hybrid: netty white ×white spotted	23.2 – 69.0	11.0	18.0	29.0	64.1	0.75	75.0	75.0	48.0
Ternary hybrid:netty white × white spotted×Silver Full leaf	35.4 – 87.8	15.0	24.0	39.0	63.3	0.79	79.0	79.0	65.0
Quaternary hybrid:nettywhite × white ×spotted×SilverFullf×resistant	29.4 – 87.8	15.0	24.0	39.0	63.3	0.79	79.0	182.0	100.0

Every number in table represents the average of three replicates in three sites.

There was a difference between the appearance and the real saving period belong to more than reason, since the appearance period the longer the greater chance of injury, and the insects may discover its secret if the period elongated and not feared, so the change in the form of characters collected in hybrids (as it sequence changes from one form to another through a time organizer of the work of controlling genes) is one of the necessities of sustaining its impact in order to not easily discover by insects and be less efficiency, In addition, each form or character have a functioning efficient of their own (ratio between the saving period and the appearance period), which descended up appearing period in the pure lines, where the highest being the efficiency of the netty white that appears as a net, so insects might saw as a spider net and so repel from it and that raising its saving efficiency to 94.4%, then the full silver which might seemed like reflective mulches coated in silver, which was found that it alienated insects and delayed their visit to the plant (2, 4, 7, 9, 10) and thus its efficiency reached 92.8%, then the white spotted that appears as bright spots which might cluttered vision in front of the insects and did not saw the green color of the leaves clearly or it alter the flight behavior of aphids as (Kring, 1972) was found , but its efficiency had declined to 76.6% for the length of its appearance, as well as in hybrid the best efficiency was the binary hybrid (full silver × netty white), amounting to 84.1% because it combines two competent forms, and

it had dropped to 63.3% in the ternary hybrid (netty white × white spotted × full silver) for being the longest appearance period. The saving efficiency from virus vectors insect injury (which represents the degree of saving multiplied the character efficiency) has also varied depending on the form of increase coloration. The character efficiency was on the contrary has increased with the appearance to range between 46.0 - 65% in pure lines and the white spotted was the best because it was the longest period, and increased in hybrids to range between 70.0 -79.0 % and the best was the ternary hybrid (netty white × white spotted × full silver) because of its longer appearance.

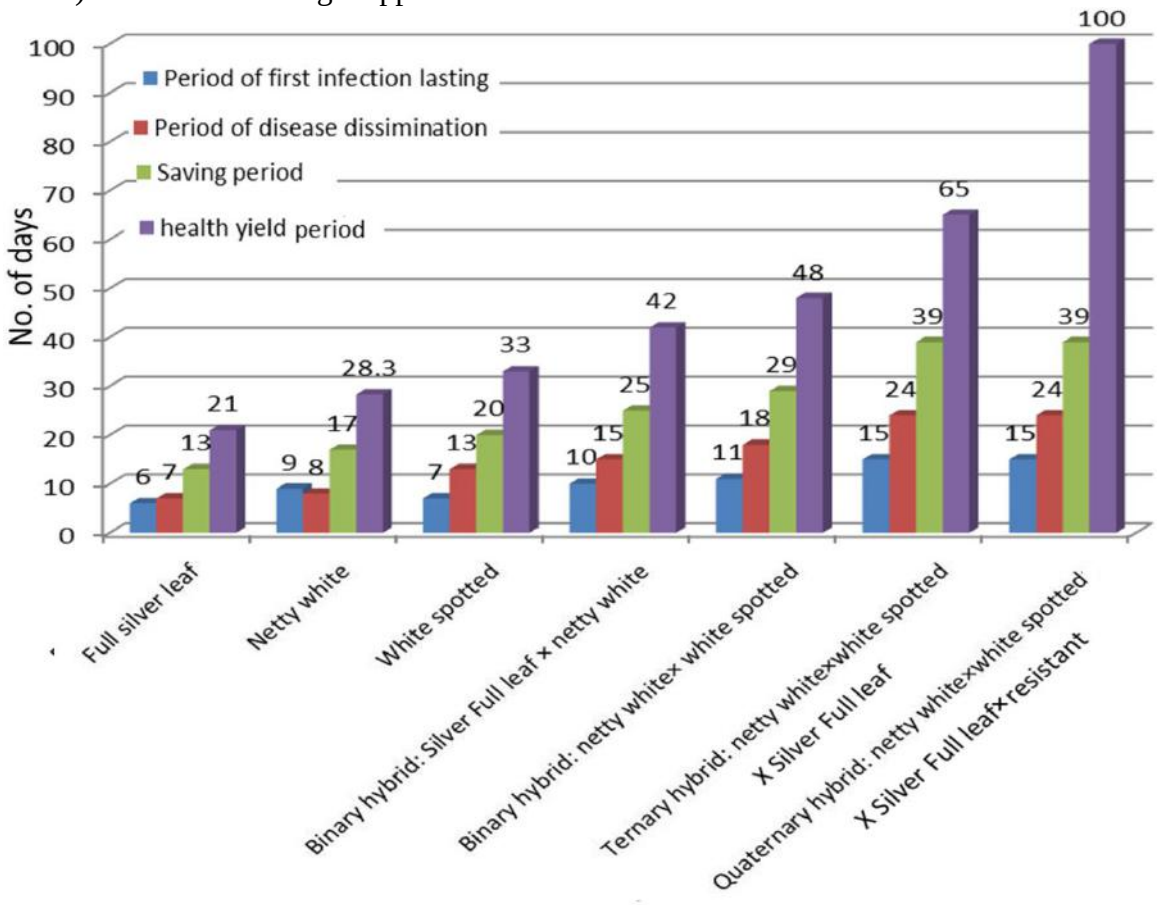

Fig .2. Periods of lasting, dissemination, saving of infection and health yield in different coloration form and their increasing with the number of form in hybrids

These ratios of efficiency of characters and saving reduce the efficiency of saving and therefore the period of saving if they were less than the maximum value 100%. Beside that there was a natural delay period to infect comparative plants deducted from saving period to obtain the real value of saving of each coloration form resulting in reduced appearance period and so saving degree and thus the saving efficiency of the form. It is clear from these results that the more time appearing the less character efficiency, but on the contrary, the saving efficiency has increased up period to appear. This reflected on the rate of health fruiting production in pure lines and hybrids (except quaternary hybrid) ranging between 25% to 65%, or between a quarter and two-thirds of the total yield or overall productivity period amounting to 60 days approximately, while the saving period has ranged between 13.0 to 39.0 days, which also covered a quarter to two-thirds of the overall productivity period, that if the injury coming agree with the beginning of the fruiting production and not before or after (this little obtain) and saving efficiency must be of 100%, but this efficiency has been ranged between 46.0 to 79.0%. In other words there was a decrease in the saving period of approximately one fifth to half, though, the health production period have increased and surpassed the real saving

period as there was an increase not decrease in saving period that covering the time difference between the two periods. This increase in the period of health production due to that the symptoms of injury not seen on fruits or the fruits were not deformed immediately after the plant injury, and might late for few days more than a week. Therefore, the real values of the saving period, degree and efficiency, or periods of health production of every coloration form in pure lines and hybrids were calculated to avoid these differences.

It can be seen from the foregoing that although of the extension of saving periods to the end of plant life through hybridization between pure lines of the coloration forms, but the low efficiency of characters and saving prevented access of the plant saving from insect injury throughout their life, especially through the intensification of virus infection or increase the density of viruleferose insect or appropriate environmental conditions for both as (Shiffriss, 1981, 1982) found , so to reach this object it has remained the needing to resistance strains to enter with saving strains, and that what has been done in quaternary hybrid (netty white × white spotted × full silver × resistant strain), which included three coloration forms plus resistant strain, that has a resistance efficiency of 83.0%. Thus the overall efficiency of this hybrid or the prevention efficiency as we called that combines the saving efficiency and resistance efficiency becoming 182\200, so they complete each other, whereas this resistance gene does not function but only when the existence of virus infection, where this strain induced resistance lead to healing plants shortly after the injury, but when injury repeated in frequent intervals, it prolongs the recovery period, and leading to symptom appearance on fruits, however, the presence of saving with resistance characters reduce insect injury repeating, a result that led to save plant from virus infection throughout the period of their life, except a short period between their infection and recovery, however the fruits remained intact 100% through the production period for non-injury symptoms appearance. This was a very major cognitive to collect genes of saving or resistance, or both in the construction of a hierarchy of resistance to plant diseases.

Conclusion

Appearance period of various coloration shape of leaves in squash differed from one format to another in pure lines and in their hybrids, the work of coloration character was marked with several properties by existence with some others in hybrids, notably the independence of the controlling genes, where the shape or character has independently appeared and every form, including kept a period of appearances own it took a solo in pure lines, where the appearance of each form were not affected by the existence of others such in the hybrids, on the contrary appearing periods were doubled by adding the period of each format to the other interred with it in this hybrid, as well as the sequencing appearance of forms, where there have been a succession appearance of coloration form and did not all appear at once, and a constant succession started its with full silver, then netty white, and then spotted white. Moreover there was a coordination between the controlling genes of traits as if organized chronologically or sensor controlling them and works to stimulate the appearance of following form shortly before disappearance of its predecessor, or as if the concentration of the stimulator is controlling this process which is which stimulates specific character appearance sequentially and constantly with its predecessor, as well as having a short interference between characters, where the character appear after a brief overlap with that preceded it, as well as with that followed. In other meaning that these traits covering each other for a short time according to the appearing sequence, where the full silver covers netty white, and netty white covers the white spotted, and this added for every two sequential coloration forms an extra period of survival ranged between 2-3 days as a period of overlap between them. This had been got benefit by the communication between coloration characters, and not gets a time gap between two sequential forms appearance that may allow access injury of virus vector insects. That

may explain that the genes had worked independently and continuously. More over these features have been obtained with economy of energy, where the genes controlling these traits did not work at the same time but only for a short period interfere of sequential characters, and if they work together that would costs plant cells extra energy without added benefit, whereas the characters would covering some others, appear one and disappear two with three character energy consumption, this did not happened and the characters appeared one after one, and so got benefit by the extension of character appearance periods longer durations and so lengthened the term of saving in hybrids. More over the sequential changes of shapes prevent their secret to be discovered by insects, and those utmost to be of hybridization, otherwise that are considerably less saving and efficiency of this process.

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RESPONSE OF MAIZE LINES TO TWO SULFONYLUREA AND TRIKETONE HERBICIDES

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Abstract

Herbicide application in some cases could lead to plant damage and yield loss. This problem is especially often in maize seed crop. In Serbia, herbicides are not registered for use in maize inbred lines. So, safe herbicide application requires maize lines sensitivity testing. The aim of this study was to test five maize inbred lines to sulfonylurea and triketons herbicides.

Three year experiment on the experimental field of the Maize Research Institute Zemun Polje, Belgrade, Serbia. Rimsulfuron and foramsulfuron as sulfonylureas, as well as mesotrione and topramezone as triketone herbicides were applied in recommended and double dose (dose for hybrid maize). Visual plant demages, plant fresh mass and grain yield were measured. Obtained data was statistically processed by ANOVA and differences between means were tested by LSD test.

Sulfonylurea herbicides caused higher plant damages compared to triketons. Injury symptoms were followed with decrease of fresh matter content, especially 2-3 weeks after herbicide application. Double dose of foramsulfuron in higher percentage decreased fresh matter on three maize lines. Sulfonylureas decreased grain yield in four lines. On the other hand, triketons induced slightly injuries according to first evaluating, while no injury symptoms were recorded during second evaluation. Mesotrione and topramezone did not influence on fresh mass and grain yield of maize lines.

Key words: *maize lines, sulfonylureas, triketons*

Introduction

One of the most profitable activities in agriculture is seed maize production. Every production have negative effects that can limit production. In seed maize one of the most important pests are weeds. Due to homozigous, maize lines often have a smaller hatibus with uneven growth than hybrid maize. Those conditions create a microclimate in seed maize where weeds are best adapted (Stefanovic et al., 2007). If weeds are not controled, higher percentage of grain yield can be lost. Homozygosis of maize linis carries other negativness, like higher susceptibility to various biotic and abiotic factors. Herbicides are first on the list that can negatively effect maize lines (Stefanović et al., 2010). So, on one hand we need to apply herbicides to control weed in maize lines crop, while on the other hand we need to safe apply those herbicides to prevent possible crop demages and injuries. Herbicides in Serbia are not registrated for use in maize seed production, so companies that product seed maize need to test their own genotypes.

Herbicides can negatively effect by growth delay, reduction in plant leaf area, plant height, leaves deformations, and in the worst case by yield loss or totaly plant demages and death. Carvalho et al., (2007) concluded that if plant are able to overcome herbicide application – it is temporary stress, while in case they can not overcome herbicide application – it is permanent stress. Permanent stress is mostly associated with greather plant demages, reducing grain yield or complete plant destruction, where a plant death is occured.

The aim of this study was to test five maize inbred lines to sulfonylurea and triketons herbicides.

Material and Methods

Three year field experiment (2010 – 2012) was set up on chernozem soil type in experimental field of the Maize Research Institute „Zemun Polje“, Serbia (44° 52´ N, 20° 19´ E, 18 asl). Five maize lines, parental components of commercial ZP hybids were tested to rimsulfuron and foramsulfuron as sulfonylureas and mesotrione and topramezone as triketons. Each herbicide was applied in two doses – recommended and double for hybrid maize. Herbicides were applied when maize lines had 5-6 leaves, what is 15-16 leaf stage according to BBCH scale. The four-replicate trial was set up according to split-plot arrangement. The main plots encompassed one 10 m row of each maize line in four replication, while subplots included herbicides and a control – without herbicides application.

Visual plant demages were estimated two times: in period of 2-3 weeks after herbicides application and 2-3 weeks after firts estimation. Visual demages were estimated according EWRC scale (0 - no plant demages and 9 – planth death; Feldfersuche Manual, 1975). For measuring plant fresh matter content, plant samples (four plant per replication) were collected also two times: first in the same period as first visual estimation and second in maize flowering stage. At the end of the vegetation cycle grain yield was estimated and calculated at 14% of moisture. Obtained data was statistically processed by ANOVA and differences between means were tested by LSD test.

Meteorological data for experiment was presented in table 1. In first vegetation cycle weather conditions were good for maize production with good distribution of percipitacion. On the other hand, second and third year had a lower quantity of percipitation with a drought period in third year.

Table 1.Precipitation and average air temperatures for the period April-September

Months	Precipitation (mm)			Temperature (°C)		
	2010	2011	2012	2010	2011	2012
April	44.0	14.9	64.2	13.2	13.4	14.45
May	64.1	89.6	66.4	17.5	16.8	17.9
June	167.3	26.2	17.5	21.0	21.5	24.56
July	35.6	44.0	30.7	23.2	23.3	27.08
August	68.2	66.0	5.8	23.1	23.9	26.21
September	68.0	32.6	26.0	17.6	21.6	22.14
Average	447.2	273.3	210.6	19.3	20.1	22.05

Results and Discussion

All tested lines had higher damages with application of sulfonylurea herbicides. First two lines had moderate to tolerable damages in first visual estimation, while other lines had light to very light demages. In second evaluation only L1 had significant demages with sulfonylureas. Other lines did not had significant demages. Triketones herbicides also did not cause significant damages in both evaluation (table 2). According to Malidža (2007) and Stefanović et al., (2007) introduction of sulfonilurea herbicides have led to lower crop tolerance and higher susceptibility of certain maize lines. In our experiment, first two lines which belong to early maturity group, have been shown as sensitive.

Table 2. Visual damages of maize lines (first and second evaluation)

1st eval.	Herbicides								
	Mesotrione		Topramezone		Control	Rimsulfuron		Foramsulfuron	
Lines	RD	DD	RD	DD	-	RD	DD	RD	DD
L1	1.33	1.83	1.83	1.75	1.00	4.42*	5.58*	5.58*	6.42*
L2	1.42	1.83	1.67	2.00	1.00	3.08*	3.67*	3.50*	4.58*
L3	1.50	1.75	1.75	1.83	1.00	2.42	2.25	2.58	2.75
L4	1.58	1.75	1.67	1.75	1.00	2.50	2.25	2.83	2.42
L5	1.67	1.50	1.42	1.50	1.00	2.33	2.17	2.50	2.25
2nd eval.	Herbicides								
	Mesotrione		Topramezone		Control	Rimsulfuron		Foramsulfuron	
Linija	RD	DD	RD	DD	-	RD	DD	RD	DD
L1	1.17	1.50	1.17	1.50	1.00	4.33*	4.67*	4.92*	6.08*
L2	1.00	1.33	1.25	1.33	1.00	1.33	3.33	1.33	4.42*
L3	1.17	1.17	1.17	1.17	1.00	1.33	1.50	1.58	1.50
L4	1.00	1.17	1.00	1.17	1.00	1.33	1.33	1.33	1.50
L5	1.25	1.17	1.17	1.17	1.00	1.33	1.67	1.33	1.67

L1 - PL38; L2 - PL39; L3 - L335/99; L4 - L375/25-6; L5 - L155/18-4/1 RfVg; RD – recommended dose; DD – double dose; * - significant at 0,05 level, ($p<0,05$).

Fresh matter content varied also only with application of sulfonylureas. First two lines, that had the highes injuries, had significantly lower fresh matter content at all treatments with sulfonylureas. Double dose of foramsulfuron significantly reduce fresh matter content in L3 in first evaluation. Other lines did not have significant reduction with herbicide application, while triketons also did not cause plant fresh matter reduction. Similar results were obtained in second evaluation, where first two lines did not recover from herbicide application. Only recommended dose of rimsulfuron did not cause fresh matter content in L3. Triketons did not cause variation in second evaluation (table 3). Green and Urlich (1994) stated that variation of fresh matter content is a good indicator of maize susceptibility to herbicides. Brankov et al., (2015) stated that application of rimsulfuron and foramsulfuron caused reduction in fresh matter content in maize lines.

Table 3. Fresh matter content of maize lines (first and second evaluation)

1st eval.	Herbicides								
	Mesotrione		Topramezone		Control	Rimsulfuron		Foramsulfuron	
Lines	RD	DD	RD	DD	-	RD	DD	RD	DD
L1	26.13	30.45	28.28	25.67	21.79	15.88*	15.01*	16.13*	11.06*
L2	32.23	32.06	32.78	35.11	35.24	29.17*	27.18*	29.25*	22.42*
L3	34.40	36.00	28.62	37.51	29.45	28.23	27.43	26.90	25.63*
L4	36.88	38.38	34.95	38.35	39.24	37.26	38.71	38.10	38.26
L5	18.75	19.09	19.36	19.16	20.48	19.48	18.60	19.27	18.09
2nd eval.	Herbicides								
	Mesotrione		Topramezone		Control	Rimsulfuron		Foramsulfuron	
Linija	RD	DD	RD	DD	-	RD	DD	RD	DD
L1	397.9	398.7	401.4	411.3	407.6	303.4*	279.4*	295.2*	218.3*
L2	521.5	519.5	510.4	500.2	520.1	407.7*	415.7*	436.0*	356.0*
L3	700.9	719.1	711.9	714.4	707.0	699.3	531.4*	615.6*	622.1*
L4	841.9	832.0	840.5	852.2	852.6	844.2	833.4	830.2	843.7
L5	502.2	478.2	488.3	479.7	481.9	501.5	486.1	487.2	487.6

L1 - PL38; L2 - PL39; L3 - L335/99; L4 - L375/25-6; L5 - L155/18-4/1 RfVg; RD – recommended dose; DD – double dose; * - significant at 0,05 level, ($p<0,05$).

Maize grain yield was significantly lower only with sulfonylurea herbicides. First two lines, L1 and L2, at all treatments with sulfonilureas had significantly lower grain yield. Double dose of foramsulfuron decrease grain yiled of L1 down to 60%. Double doses of rimsulfuron and foramsulfuron caused significantly grain yield reduction in L3 and L5. Grain yield of L4 did not vary with herbicide application. On the other hand, mesotrione and topramezone did not affect maize lines grain yield (table 4). Brankov et al., (2012) stated that application of sulfonylurea herbicides cause significant grain yield reduction. Similar results were obtained by Dragičević et al., (2011).

Table 4. Maize grain yield (t/ha)

	Herbicides								
Lines	Mesotrione		Topramezone		Control	Rimsulfuron		Foramsulfuron	
	RD	DD	RD	DD	-	RD	DD	RD	DD
L1	1.01	0.99	0.98	1.05	1.13	0.78*	0.69*	0.63*	0.51*
L2	1.92	1.83	1.82	1.95	1.90	1.59*	1.58*	1.58*	1.52*
L3	4.35	4.21	4.25	4.31	4.07	4.19	3.76*	4.26	3.63*
L4	3.97	3.93	3.97	3.93	3.90	3.64	3.82	3.76	4.02
L5	2.64	2.52	2.55	2.63	2.68	2.36	2.09*	2.44	2.15*

L1 - PL38; L2 - PL39; L3 - L335/99; L4 - L375/25-6; L5 - L155/18-4/1 RfVg; L1 - PL38; L2 - PL39; L3 - L335/99; L4 - L375/25-6; L5 - L155/18-4/1 RfVg; RD – recommended dose; DD – double dose; * - significant at 0,05 level, ($p<0,05$).

Conclusion

According to obtained data, mesotrione and topramezone showed good selectivity towards tested maize lines and these herbicides can be safely use in seed maize production. On the other hand, rimsulfuron and foramsulfuron showed lower selectivity towars tested maize lines and for their application special attention is required.

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THE FIRST REPORT OF BIPOLARIS/DRECHLERA SOROCINIANA(Sacc in sorok.) ON ANISE SEEDS IN SERBIA

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Abstract

Anise (*Pimpinella anisum* L. fam. *Apiaceae*) is important medicinal plant and a popular aromatic and spice crop. Aniseed is rich in essential oils and is used in the food industry, as well as for medicinal and cosmetic purposes. Its production has increased in Serbia in the last decade. The aniseed were collected the locality Pančevo during 2012-2013. Using conventional mycological seed testing, according to the ISTA rules, different fungal species were isolated. Monosporial representative isolate BS-1 was used for morphological and molecular identification. The fungal colonies grew rapidly in PDA media, reaching a diameter of 4 to 10cm following incubation at 25°C for 8 days on potato dextrose agar. Colonies were dark olive and slight cotton-like, totally covered by short conidiophores, with three to five black conidia in their apex. The reverse side of the colony was dark olive. Conidia size ranged from 40 to 117μm long and from 18 to 27μm wide, with 4 to 12 pseudosepta. They were usually straight (90%), ellipsoid, with round edges, rarely curved (10%), medium brown to dark brown in color. Conidiophores were between 170 to 280μm in length. The total DNA was extracted from fungal mycelium with DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The rDNA internal transcribed spacer (ITS) regions of one isolate were amplified using polymerase chain reaction (PCR) with universal fungal rDNA primers ITS1 and ITS4. The sequence analysis of the ITS region revelated that isolate BS-1 (GenBank Accession No. KR866080) shared 100% nt identity of many *Bipolaris / Drechlera sorociniana* (Sacc in sorok.) isolated deposited in the Gen Bank from different region of the word. To confirm pathogenicity, a suspension of 2×10^6 spores/ spores/ml from the 14 days old culture were used for inoculation of the seeds. The level of root necrosis was calculated after 30 days, according to the scale from 0-3. On the basis of fungal macroscopic and microscopic characteristics, pathogenicity testing, and the ITS region sequence, this fungus was identified as *Bipolaris/Drechlera sorociniana*.

Key words: *aniseed, pathogen, identifications*

Introduction

Anise, *Pimpinella anisum*, is a herbaceous annual plant in the family Umbelliferae grown primarily for its fruit which is used as a spice in cooking or as a confectionery flavouring. The leaves can be eaten in salads.

Mycopopulation of anise seed are not investigated thoroghly. Only a few fungal species have been reported in the world as anise seeds pathogen: *Alternaria alternata* (Strandberg, 2002), *Puccinia pimpinellae* (Ghoneemet et al., 2009), *Erisyphe heraclei* and *Peranospora umbellifarum* (Davis, 2004).

The mycobiota of seeds of medicinal plants is well documented, but data on fungal species associated with anise seeds missing in Serbia. Consequently, the mycobiota of anise seeds were studied in 2012 and 2013 at the cultivating plantation of the Institute for Medicinal Plants Research "Dr Josif Pančić". The aim of this research was to determine mycopopulation of anise seeds for the first time in Serbia.

Material and Methods

Seed sampling, fungi isolation and pathogenicity test

Four hundred seeds (4 trials, each with 100 seeds) were sterilized with NaOCl for 2 minutes and then rinsed with sterile water and transferred to the filter paper and PDA medium on Petri dishes (Mathur and Kongsdal, 2003). After the fifty-day incubation at 25°C, parts of the mycelia taken from well - developed colonies was transferred to the PDA in order to be further examined**.**

Morphological examination of the eight selected isolated fungi (BS-1, BS-12, BS-17 from 2012 and BS-6, BS-8 and BS-16 from 2013 year) was conducted using monosporial cultures (Dhinger and Sinclair, 1986). The following characteristics were monitored: the appearance of aerial mycelia, presence of pigmentation, the appearance of conidiophores and conidia and the manner of conidia formation on the 15 old days fungal isolates. A hundred conidia were measured in every isolate.

Identification of the isolated fungi species was based on morpho-physiological characteristics and the cultivation of the fungi tested (Bockus et al., 2010).

The pathogenicity test of six isolates was confirmed by the modified method of Molot and Simone (1967). A total of ten anis seeds, surface-sterilized with sodium hypochlorite per isolate were inoculated in the suspension containing 2×10^6 spores/ml from the 14 days old culture. The control seeds were immersed in the sterile water. The inoculated and control seeds were transferred to the pots containing a sterile organic soil in greenhouse condition. The level of root necrosis was calculated after 30 days, according to the scale from 0-3 (0- not pathogenic; 1 - low pathogenicity; 2 - moderate pathogenicity; 3 - very high pathogenicity).

DNA extraction and PCR

The total DNA was extracted from fungal mycelium with DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The rDNA internal transcribed spacer (ITS) regions of one isolate were amplified using polymerase chain reaction (PCR) with universal fungal rDNA primers ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC'3) designed by White et al. (1990). Amplification reactions (25 µl) contained 10×PCR buffer, 5 U/µl Taq polymerase (Kapa Biosystems), 0.4 mM dNTPs, 25 mM $MgCl_2$ (Fermentas), 0.5 µM of each primer and 10 ng of genomic DNA per reaction mixture. PCR was performed in a Thermal Cycler PCR system 2720 (Applied Biosystems, USA). Initial denaturation at 95°C for 2 min was followed by 36 cycles at 94°C for 1 min, 57°C for 1 min (primer annealing), 72°C for 1 min (primer extension). A final extension at 72°C for 10 min was incorporated into the program, followed by cooling to 4°C until recovery of the samples. PCR products were visualized using UV light and separated in 1% agarose gel following staining with Midori Green DNA Stain (Nippon Genetics).

Sequence analyses

Sequencing in both directions was performed on an automated sequencer (ABI 3730XL Automatic Sequencer Macrogen, Korea). Sequence generated in this study was deposited in the National Center of Biotechnology Information (NCBI) GenBank database. Sequence of the Serbian isolate (BS-1) was compared with the respective fungi sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov/ BLAST/) using the ClustalW program (Thompson et al., 1994) and MEGA5 software (Tamura et al., 2011).

Results and discusion

Analysing results of the collected anis seeds mycopopulation enabled different species from genus: *Alternaria, Phoma Fusarium, Bipolaris/Dreschlera, Epicoccum, Penicillium,*

Aspergillus and *Rhysopus* to be indentified. *Bipolaris sorokiniana* (Sacc in sorok.) (Fig. 1) was a predominant pathogen species on anes seeds, accounting for 8 and 15% in 2012 and 2013, respectively.

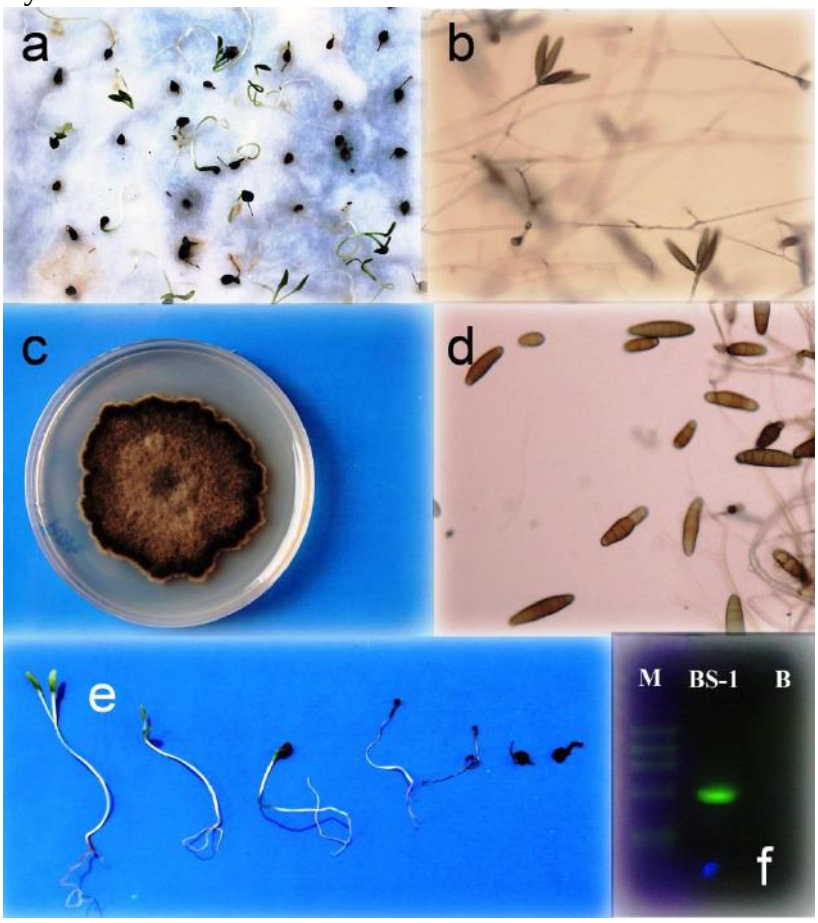

Figure 1.*Bipolaris/Drechlera sorociniana*.- a) pathogen on germinating anise seeds, b) and d) apearance of conidia, c) a colony on PDA, e) seedlings of artificially infected aniseed (left-seedling from a non-inoculated seed), f) Electrophoretic analysis of PCR products obtained using primer pair ITS1/ITS4. Lanes: M- Mass-Ruler^{TM}DNA ladder, Mix (Fermentas Life Sciences GmgH, Lithuania); 1- isolate BS-1; B- negative control (PCR mix with Rnase-free water)

Pathogenicity of tested isolates *Bipolaris sorokiniana.*

All six investigated isolates of *B.sorokiniana* caused root and leaf necrosis of seedlings (Tab.2). There were no significant differences between the isolates, but the isolate BS-1 showed the highest pathogenicity in comparison with the other five. All isolates showed a degree of pathogenicity.

Table 2. A degree of pathogenicity of *Bipolaris sorokiniana* on the anise seedlings in laboratory conditions

Isolates	Pathogenicity
BS-1	3
BS-12	2
BS-17	2
BS-6	1
BS-8	1
BS-16	2
Control	0

0- not pathogenic
1- low pathogenicity
2- moderate pathogenicity
3- very high pathogenicity

Morphological characteristics

After eight-day incubation on filter paper on the surface of the investigated anise seeds individual straight, shape conidiophores were observed measuring 170 to 280 (average 210 μm), tooped located three to five radially arranged conidia. On the potato dextrose agar medium a compact, initially olive brown, and subsequently black mycelium was formed. Conidia were usually straight or slightly curved, 40-117 x 18-27 μm in lenght, and golden brown, with 12 pseudosepta. These morphological and cultural characteristics are similar to *Bipolaris sorokiniana* already described by Ellis (1971), and Pidopličko (1977). Although the germination of conidia was mainly bipolar, lateral germination was observed sometimes.

Molecular detection and identification

Electrophoretic analysis of the PCR product showed that the fragment was of the expected length of 560-bp. In a BLAST search analysis, the sequence of the Serbian isolate BS-1 (Acc.No. KR866080) showed the highest nt homology of 100% with 16 *B. sorociniana* isolates deposited in GenBank from Italy (KJ909776), India (HM195250-60), Brazil (KF765399, KF765404, KF765404), China (KJ026137) and Canada (GU934504).

Bipolaris sorokiniana is an aggressive pathogen, transmitted by seeds that causes spot blotch, root and crown rots, node canker and head and seedling blight (Zillinsky, 1983). *Bipolaris sorokiniana* (Sacc in sorok.) is already known as causal agent of spot blotch of barley (*Hordeum vulgare*) and a common foliar pathogen worldwide. It has a wide host range and it is economically important wheat pathogen (Marthre, 1982, Nutteret al., 1985). *Bipolaris sorociniana* on oats in America secretes toxins and enzymes that destroy the plant (Agrios 1997).

This study represents the first attempt to characterize pathogens of *Bipolaris sorokiniana* in anise seeds in Serbia. Mycopopulation of anise seed is insufficiently known.

The different species from genus *Fusarium* (Pavlović et al., unpublich data) and *Alternaria alternata* **(**Stanojković-Sebić et al., 2014) have been detected on anise seeds previously in Serbia. Ghoneen et al., (2009) has reported *Puccinia pimpinellae* on anise seeds.

Conclusions

According to the cultural, macroscopic and microscopis characteristics, selected isolates from anise seeds were identified *as Bipolaris sorokiniana* (Sacc in sorok.). The representative isolates B-1 showed the highest nt homology of 100% with 16 *B. sorociniana* isolates

deposited in GenBank from Italy, India, Brazil, China and Canada. *Bipolaris sorokiniana* was a predominant pathogen of anise seed during 2012 and 2013. *B.sorokiniana* caused root and leaf necrosis of anise seedlings. To the best of our knowledge, this is the first report of *B.sorokiniana* being isolated from naturally infected anise seeds in Serbia.

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DIVERSITY OF LEAFMINERS OF PEAR IN THE REGION OF EAST SARAJEVO

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Abstract

Diversity of leafminers in region of East Sarajevo, was examined in 2011. and 2012. in intensive plantations (locations Vojkovici and Kula), in semi-intensive plantations (locations Tilava and Petrovici) and in extensive plantation (location Kasindo). In Kula, examination was done on the following cultivars: „Viljamovka“ (Bartlett/Wiliams), General Le Clerc, „Passa Crasana“, Abe Fetel and Poire de Curé.

Six species of leafminers from four families was determined. Family Lithocolletidae is presented with three species: spotted tentiform leafminer miner (*Lithocoletis blancardella* Fabricius), hawthorn red midget moth (*Lithocoletis corylifoliella* Haworth) and garden apple slender (*Calisto denticulella* Thunberg), family Nepticulidae with one: apple pigmy (*Stigmella malella* Stainton) and from the family Lyonetiidae, two species was found: pear leaf blister moth (*Leucoptera malifoliella* (Costa (1836)) and *Coleophora hemerobiella* Scopoli from family Coleophoridae.

The highest number of damaged leaves was found in the semi-intensive plantations, in localities Tilava and Petrovici, where *S. malella* and *C. denticulella* were dominated species. In intensive plantations, in the locality Vojkovici, only *S. malella* was found. In the locality Kula, except *S. malela* which was the most numerous on pear cv. the Williams, *L. blancardella* was also determined. In extensive orchards, in the locality Kasindo, the most common species was *C. denticulella*.

Keywords: *leafminers, pear, East Sarajevo.*

Introduction

Leafminers from order Lepidoptera are economically important pests in areas where pears are grown. The damage are caused by larvae that are feed on the leaf parenchyma below the epidermis, leaving empty couloir, or "mine" of different shapes and sizes. The presence of mines on leaves reduces assimilation and transpiration surface, which adversely affects the yield, fruit quality and, in general, physiological condition of plants. Importance of these pests and their negative impact on fruit production, started increasing during end of the 60´s in the XX century. Leafminers became a serious problem, particulary in plantations in most European countries (Dimic, 1964, 1968). The presence and importance of different species of isn't the same in all areas of their distribution.

In Europe, more than 20 species of leafminers from genera: *Coleophora, Leucoptera, Stigmella, Lyonetia, Lithocolletis, Incurvaria, Recurvaria* and *Calisto* were detected on the pear (Hering, 1957). The most important species on the pear are: *Lyonetia clerkella, Leucoptera malifoliella, Lithocolletis blancardella, Lithocolletis corylifoliella and Callisto denticulella* (Almasi et al., 2004). In Turkey, for example, three species was identified on pear: *Lyonetia prunifoliella* (Hubner, 1796), *Lyonetia clerkella* and *Leucoptera malifoliella* (Seven, 2006).

After *Lithocolletis blancardella* was introduce in North America from Europe, it became an important pest (Corrigan and Bennett, 1987), and a increasing problem in plant production, because of its resistance to organophosphorus insecticides, especially azinphos-methyl (Hogmire and Beavers, 1998).

In Romania, in pear orchards, the most important species are *L. blancardella* and *Lithocolletis corylifoliella* (Frasin et al., 2008; Vasian et al., 2013).

In the former Yugoslavia, a mass phenomenon leafminers occurred in the late 70´s in XX century, when they became a serious problem. For example, eight leafminer species were detected on the pear in Slovenia: *Lyonetia clerkella, Lithocolletis corylifoliella, Lithocolletis cerasicolella, Calisto denticulella, Stigmella pomella, Stigmella piri, Stigmella plagicollella, Stigmella prunetorum.* In Serbia and Croatia were registered five leafminer species: *Leucoptera malifoliella, Lyonetia clerkella, Lithocolletis corylifoliella, L. blancardella* and *Stigmela malella* (Krnjaić and Grujic, 1982). Distribution and harmfulness caused by leafminers were studied in Bosnia and Herzegovina during the 60´s, when a rapid increase in population density and damages were registered. In that period, a total of 45 species of leafminers were found, out of which in Sarajevo and its wider area 5 species was recorded on the pear: *L. corylifoliella, L. mespiliella* Frey, *Lyonetia clerkella, Cemiostoma scitella and Coleophora hemerobiella.* In that period, according to the intensity of the attack, the most harmful was *L. corylifoliella*, especially on young trees (Dimic, 1964, 1968).

The area which now includes the East Sarajevo presents a part of the Sarajevo region and there are not enough data regarding harmful leafminers on pears. The tendency of increasing the production of pears in this area in the last 10 years, resulted in establishment the new, intensive plantations. Having in mind that leafminers are economically important pests of pear, the aim of this study was to register their diversity, to detect the most numerous species, as well differences in their number, both in plantations where different growing tehniques are aplied, as well between the cultivars.

Materials and methods

The examination was realized in 2011. and 2012. in the field (locations Vojkovici, Kula, Tilava, Petrovici, Kasindo) and in the laboratory of the Agricultural faculty in East Sarajevo. Samples of pear leaves were taken four times from June to the end of October for leafimers presence in all inspected locations. Every time 100 randomly sampled leaves were taken from pear trees (total of 400 leaves per each locality), except in the locality Kula, where 400 leaves of each variety was sampled.

All sampled leaves were observed in laboratory for presence of mines as well the type of mines. Number of damaged leaves was calculated. The before adult stadium of insects who located in the mines, were reared to adult stadium. The rearing was in plastic containers and Petri dishes with a layer of cellulose wadding on the bottom of which is occasionally yeast in order to maintain the freshness of the list. Determination of species was based on morphological characteristics of the adults and shapes of mines, folloowed by keys and appropriate entomological literature (Balachowsky 1966, Hering, 1957).

Results and discussions

Two-year research of diversity of leafminers on pears in the area of East Sarajevo, resulted in six detected species from four families. From family Lithocolletidae, three species were found: *Lithocoletis blancardella* Fabricius, *Lithocoletis corylifoliella* Haworth and *Calisto denticulella* Thunberg. From family Nepticulidae results of our examination showed presence of *Stigmela malella* Stainton, from family Lyonetiidae it was *Leucoptera malifoliella* (Costa (1836)), while *Coleophora hemerobiella* Scopoli which belongs to family Coleophoridae, was found only in certain leaves. The number of damaged leaves, varied by the species of leafminers and inspected cultivars (Table 1).

Regarding the total number of 323 damage leaves, found in all inspected localities, the most numerous species was *S. malella*, whose mines were registered in 125 leaves or 38,69%, followed by *C. denticulella* (97 leaves or 30,03%) and *L. blancardella* with 74 damaged leaf

or 22,91%. The lowest number of damaged leaves was caused by *L. corylifoliella* (20 leaves or 6,19%) and *L. malifioliella* (7 mined leaves or 2,16%).

Table. 1. The number of mined leaves on pear by species of leafminers and localities

Species of leaf miners / Locality	*Leucoptera malifoliella*		*Lithocoletis blancardella*		*Lithocoletis corylifoliella*		*Stigmela malella*		*Calisto denticulella*		Total number of mined leaves in localities
	Number of mined leaves	%	Number of mined leaves	%	Number of mined leaves	%	Number of mined leaves	%	Number of mined leaves	%	
Vojkovici	**-**	**-**	**-**	**-**	**-**	**-**	**13**	**3,25**	**-**	**-**	**13**
Kula	**-**	**-**	**11**	**37,93**	**-**	**-**	**18**	**62,06**	**-**	**-**	**29**
Tilava	**7**	**5,69**	**26**	**21,13**	**9**	**7,32**	**45**	**36,59**	**36**	**29,26**	**123**
Petrovici	**-**	**-**	**18**	**23,68**	**11**	**14,47**	**21**	**27,63**	**26**	**34,21**	**76**
Kasindo	**-**	**-**	**19**	**23,17**	**-**	**-**	**28**	**34,15**	**35**	**42,68**	**82**
Total number of leaves mined by species	**7 (2,16%)**		**74 (22,91%)**		**20 (6,19%)**		**125 (38,69%)**		**97 (30,03%)**		**323**

The highest number of mined leaves was found in the locality Tilava were 123 leaves in total were damaged or 30,75%. Out of total number of leaves with „mines“ from this locality, it was found that the most abundant was *S. malella* which damaged 45 or 36,59% leaves, *C. denticulella* with 36, or 29,26%, and *L. blancardella* with 26, or 21,13% of damaged leaves. Leaves were damaged the least by *L. malifioliella*, 7 or 5,69%, while *L. corylifoliella* damaged 9 or 7,32% leaves.

In the locality Petrovici, out of the total number of examined leaves, 76 leaves or 19% were damaged.

The most abundant species was *C. denticulella* which damaged 26, or 34,21%, and is followed by *S. malella* with 21 or 27,63%, *L. blancardella* with 18, or 23.68%, while the *L. corylifoliella* was of lower presence with 11, or 14,47% damaged leaves.

In locality Kasindo, the most abundant species was *C. denticulella* with 35 or 42,68% damaged leaves, and *S. malella* with 28, or 34,15%, while the lowest number of damaged leaves was caused by *L. blancardella* (19, or 23,17%).

In the locality Vojkovići, only *S. malella* was found and damaged 13, or 3,25% leaves.

In the locality Kula, 29 damaged leaves were registered. Out of these, *S. malella* caused damage on 18, or 62,06% leaves, with the highest number of mined leaves on cv. Williams (8), then the cv. „Passa Crasana“, 4, while on the cultivars, Abe Fetel and General Le Clerc 3 damaged leaves were found. Apart form *S. malella*, it was also detected presence of, *L. blancardella* which damaged 11 or 37,93% leaves. Of these, on the cultivars Wiliams, was found 5 mined leaves, cv. Abate Vettel 4, and the cv. of General Leclerc, 2 leaves.

As results of the analysis on presence of leafminers in different locations, it is evident that the most abundant species is *S. malella* which is also dominant species in many European

countries since 1950. in apple orchards. On the other hand, there is no enough information about it's harmful effects on the pear. Besides *S. malella*, numerous species was *C. denticulella* which is not considered economically important pest and is mainly present in the extensive orchards according to the literature data (Almasi, 2004). Literature data about the rare presence of *L. malifoliella* in Europe and Asia (Jenser et al., 1999; Radoslav et al., 2001; Magud, 2002) are in correspodence with our results because this species was represented only with 2,16% in comparision with the other species of leafminers.

Conclusion

In pear orchards in the area of East Sarajevo, six leafminer species were found: *Lithocoletis blancardella, Lithocoletis corylifoliella, Calisto denticulella, Stigmella malella, Leucoptera malifoliella* and *Coleophora hemerobiella*.

In relation to the total number of mined leaves in all localities, the most numerous species was *S. malella*, which was found on 38,69% leaves and the least numberous was *Leucoptera malifoliella* and is detected only in 2,16% mined leaves.

In the intensive orchards, in the locality of Kula and semi-intensive orchards in the locality Tilava, *Stigmella malella* was the most abundant, as well the only identified species in intensive plantation in the locality Vojkovici. In semi-intensive orchards in the locality Petrovici and extensive plantation in locality Kasindo, the most abundantspecies was *Calisto denticulella.*

The highest number of mine were caused by *Stigmella malella*, established at the cv. Williams, and the lowest number on cv. Abe Vetel and General Le Clerc.

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ORGANIC AGRICULTURE

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ECONOMIC ANALYSIS OF OIL PRODUCTION FROM ORGANIC LAVENDER

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Abstract

Lavender yields of depend on the age of the plantations. It takes 8-10 kg of fresh flowers to gain one kilogram of pure dry flowers. Biannual yields of fresh lavender flowers range from 600 to 800 kg/ha or 3-6 kg/ha of aromatic oils. Lavender flowers on an existing four year old plantation can achieve yields of fresh flowers which range between 3,500 and 4,000 kg/ha, or 20-26 kg/ha of aromatic oils. The yields of fresh hybrid lavender flowers on a two year old plantation range from 1,000 to 1,500 kg/ha or 10-15 kg/ha of aromatic oils. Plantations older than four years can produce flower yields of 5,000-7,000 kg/ha. Seed yields are between 200-300 kg/ha.

The subject of analysis in this paper is the profitability of organic lavender oil production grown on one hectare an area. The aim of the research is to determine economic viability of lavender over the profitability of oil production using production results On average, from 2008 to 2012, the analyzed production of lavender oil and one hectare lavender yields the total production costs which amounted to 5.926,23 €. Gross earnings amounted to 3.810,52 €. After the representation of the total costs, costs of materials participated with 20.65%. The calculated cost of Maillet oil hybrids is on average 60.00 €/kg. There is a total profit of 1.018,77 €/ha, which means that organic farming and production of lavender oil is a highly profitable business.

Keywords: *lavender oil, variety of Maillette, costs of production, profit*

Introduction

Thanks to its geographical position, climate and soil, respectively, the territory of Bosnia and Herzegovina (BH) is suitable for cultivation of agricultural crops, among others, medicinal, aromatic and spice plants. Land, usually unfavorable because of its physical, chemical, biological and other characteristics – for example arable crops would not achieve satisfactory production and economic results, can be used for the cultivation of medicinal and aromatic herbs. Total production of lavender oil depends on the activity, capacity and number of flower parts containing oil (calyx) and the number of flower heads, branches and the number of plants per hectare. Therefore, managing processes of plants for a maximum contribution of oil need to focus on the flower and growth, so as to reach the maximum number of healthy plants with thick branches and leaves per hectare. The soil and climate conditions greatly affect the yields and quality of the oil. The soil should therefore be analyzed regularly; it is recommended every 3 to 4 years, and if necessary, make appropriate improvements. The maximum potential production and quality of the oil depends on the choice of the variety and its genetic potential. Lavandins have higher oil yield than L. Angustifolia, which is why most lavender oil in the world is made from lavandin.

In BH, lavender was previously grown on plantations covering large areas and thus there are no adequate data on yields. Among the first scientists who described the lavender was Coulper (1952), and in BH it was Devetak (1949). The research by Peco (2009), established the biological productive traits of hybrid lavender grown on the principles of organic production; yields and lavender flower oil obtained by distillation, the differences that exist in

the respective characteristics of a hybrid, as well as the differences in the characteristics of two hybrids. Accordingly, the subject of the research is establishing the economic effects of the cultivation of lavender.
The aim of the research is to determine economic viability of lavender over the profitability of oil production using production results.

Materials and Methods

Authors used data from analytical and accounting records of lavender growers in the municipality of Mostar (BH) for this research. In addition, different websites and various publications from domestic and foreign authors have been consulted. By applying statistical calculations and methods, significant economic indicators and the evaluation of the economic viability of production are determined. The results are presented in tables and graphs along with appropriate comments.

Results and Discussion

Biological and technological properties of lavender

Lavender has a long history of cultivation worldwide. Lavandula (common name lavender) is a genus of 39 known species (https://www.en.wikipedia.org/wiki/Lavandula). This is a perennial plant. According to Jancic (1990), lavender grows in the form of a hemispherical bush height and diameter of 100 to 150 cm. The root is branched and woody. The tree is branched and bushy with many branches. Lavender leaves are flat, elongated and lanceolate, 0.5 cm wide and 5 cm long. Young lavender plants are of gray-green color and age green. The flowers are grouped in earlike blossoms. The color of flowers varies from deep purple, lilac, blue, pink to white, depending on the hybrid. One inflorescence has 6 to 10 flowers. Lavender blooms from July to August. There are about 20 different hybrid lavender plants. The name lavender comes from the Latin word "lavare" which means "to wash" (Houdret, 1999).
The scale of production of medicinal and aromatic herbs, including lavender, in Europe and the world has undergone significant changes in recent years. There was an intensification of production in order to increase labor productivity and production volume, as well as to improve product quality. Production of organic lavender has derived from such a concept. Organic production currently represents the best compromise between the demands of agriculture (quantity and quality) and the requirement to protect the environment and produce food that is safe enough and health-wise for consumers (Milic and Lukac-Bulatovic, 2013).
The selection of varieties in the production of lavender is an important issue to be solved before plantation. Long period of exploitation and a large number of hybrids of different economic and biological characteristics stress out even more significant problems for the proper resolution of assortments. Mistakes made in the selection of new cultivars can later be difficult to remove, and if there are certain possibilities, it is necessary to allocate additional financial resources (Milic and Radojevic, 2003). In the first year, in late May, lavender is pruned to 8 or 10 cm, to form a dense compact shrub. During the second year it is re-trimmed to a height of 15 to 18 cm. The old lavender plantation after six to eight years is rejuvenated by cropping plants during dormancy at a height of 10-15 cm by special machines. The plantation will give a full yield year after pruning. This way, the crop can be rejuvenated two to three times during the lifetime of proper lavender. Old branches of hybrid lavender that do not provide new bursts rejuvenate as proper lavender (Oliver, 1996).

Photo 1: Lavender Maillete

Flower stalks are cut to the height below the first pair of leaves. Improper pruning reduces the quality of the plant. For smaller areas, reaps are most usually manual while on larger areas special machines that can be drawn or self-propelled are used.
Lavender inflorescences are harvested at the stage when 50-75% of flowers are in bloom. One worker can manually collect 100-150 kg lavender flowers or 300-500 kg of hybrid in ten hours. Special machines collect crop off of 0.1-0.3 hectares in an hour. After harvesting, inflorescences are dried at ventilated places in the shade. Before winter, shrubs are cleaned of subsequently grown or overly ripen blossoms.
Lavender is grown for aromatic oils. According to Dorfer et al. (1990), the first distillation of aromatic lavender oil in Europe was performed in Frankfurt in 1582. The oil is obtained by distillation from the flowers of Lavandulae Flos (formerly Flores Lavandulae). Lavender flowers contain 0.6-1.4%, while hybrid 0.9-5% of essential oils and about 10% of tannin. Aromatic oil - Lavandulae Aetheroleum (formerly Oleum Lavandulae) is colorless and of bitter taste. The main constituents of the essential oil are: linalyl acetate (35-62%), linalin, geraniol, hydroxycoumarin and others. Lavender has a broader application in home medicine for problems with headache, appetite, bloating, in repulsive reaction to food, in dizziness, nerve problems, gall bladder, problems with the liver and spleen.
As for the aromatic oil, lavender is said to be the "mother of the oils." It has a sweet, honey-floral scent. It is mild and does not irritate the skin. Any packaging must accurately say the Latin name Lavandula Officinalis or synonyms Lavandula Angustifolia or Lavandula Vera. Unfortunately, some producers sell counterfeit lavender oil under this Latin name. In addition to written Latin name, sellers possess a chemical certificate. The detailed chemical analysis must be made for every quality aromatic oil on the market. Such an analysis easily reveals retailers and distributors who sell counterfeit lavender oil.
The yields per unit area in the plantation production of lavender significantly dependent on natural conditions, silvicultural form, selected varieties, applied agricultural technology etc. Their incompatibility can cause yield reduction and the achievement of unfavorable economic results. Yields depend on the age of lavender plantations. According to *Tanovic (2002),* full yield is achieved only in the fifth or sixth year of cultivation.

Photo 2: Condenser for the production of lavender oil

8-10 kilograms of fresh bloom are needed for 1 kg of pure dry bloom. Yields of two year old fresh lavender blooms range from 600 to 800 kg/ha or 3-6 kg/ha of aromatic oils. On older plantations, up to four years of age, yields of fresh blooms range between 3,500 and 4,000 kg / ha or 20-26 kg / ha of aromatic oils. Yields of blooms of two year old fresh hybrid lavender range from 1,000 to 1,500 kg/ha, or 10-15 kg/ha of aromatic oils. On older plantations, up to four years of age, yields of 500-700 kg/ha of aromatic oils can be achieved.
McNaughton (1994) gives a special place to lavender and its use. Proper lavender oil, made of all kinds of lavender, has a very calming effect. It helps with nervousness and insomnia, and relaxes tense muscles after a day of work, stress and physical activity. Aromatic oil of broadleaf lavender (L. Spica L. Latifolia) has a mild smell, a refreshing touch, and compared with aromatic lavender oil, it smells less sweet. This aromatic oil has the most beneficial effect on skin. It has an excellent regeneration effect on oily, dry and damaged skin. Oil from broad lavender is the most effective on mild burns.

Economic results of lavender growing

The production value is the market value of the products obtained at the farm during a business year. As for the reference period of 2008-2012, the yields of Maillete hybrids oil stood on average at 1,736.25 kg or 115.75 kg/ha. The economic result of the production unit depends on the amount of cash value that is achieved through the realization of products and the amount of the costs. The greater the positive differences between these values, the more favorable economic results are of the operations. Due to its production and technological features of modern plantation, cultivation of medicinal and aromatic plants should be a highly intensive production based on high investments of capital and labor per unit of production capacity. The basic requirement for successful production is the achievement of economic optimum, from both points of view the scope and structure of production, and within the end from the investment point of view.
As for the period of 2008-2012, in the analyzed production of lavender, overall production costs on average amounted to € 5,926.23/ha (Table 1). As for the total costs, variable costs (fertilizer, fuel and other materials) account for 53%. The calculated cost price is approximately 60.00 €/kg. The profit amounted to 1,018.77 €/ha, which is very acceptable.
In terms of competition, the goal of every manufacturer is to offer, not only a greater quantity, but also lower costs. Manufacturers go for better financial result, which imposes the need for a reliable prediction, monitoring and comprehensive analysis of the components of costs.

Table 1: Economic indicator of Maillet sort in the period of 2008-2012

I n d i c a t o r s	*Amount (€/ha)*	*The structure of the total cost (%)*
1. Value of production	*6,945.00*	-
2. Variable cost	3,134.48	53.00
3. *Gross margin (1.-2.)*	*3,810.52*	-
4. Fixed cost	2,791.75	47.00
5. Total cost (2.+4.)	*5,926.23*	100.00
Profit (1.-5.)	***1,018.77***	-

As for the economic production of lavender, it is expressed through the relationship between the output and the value of the funds spent and the work or the costs of production. If the cost effectiveness ratio is greater than one, we have a more economical production and vice versa. Since the coefficient of economy of production in the examined period is 1.16, it shows that the production of Maillet lavender is profitable activity.

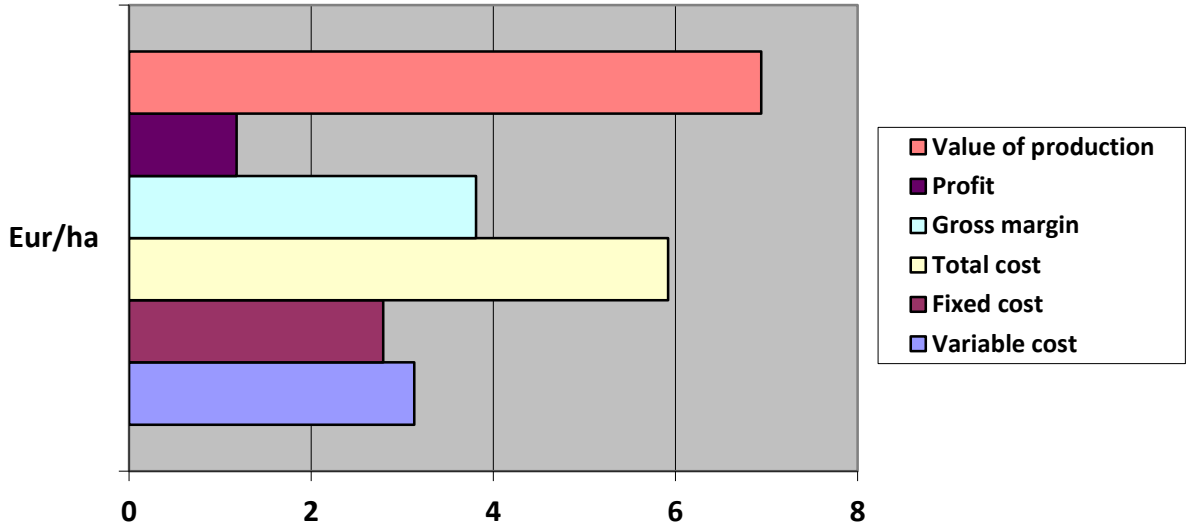

Chart1: Graphic indicators display of oil production in the (000) EURO per 1 ha of lavender oil.

One of the indicators of economic efficiency of production is also the profitability of production. The rate of return indicates the amount of profit for every 100 € of the market value of production. Determined profitability for the production of Maillet hybrid amounted to 16.56 €.

Conclusion

Project of lavender plantation started in 2001 in the research company and it brought along the latest technology and methods in lavender growing. It was necessary to implement a lot of knowledge, experience and funds. One of the many requirements is to provide security of products for the final consumer. The company management certifiers the production at the world-famous certification company Soil Association, which provides the opportunity to access the most demanding consumers. The calculated cost price of Maillet hybrids oil is, on average, 60.00 €/kg. Recorded total profit on a 15 ha plantation is € 5,926.23, which is very

acceptable. Production efficiency is 1.16 which indicates that the production is a profitable activity. The profitability of Maillet hybrid lavender amounted to 16.56 €.

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IMPACT OF VARIOUS HERBAL EXTRACTS ON YIELD OF LETTUCE (Lactuca sativa)

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Abstract

The paper presents results of a large-scale experiment dealing with the impact of various herbal extracts on yield of lettuce (*Latuca sativa*), conducted at the laboratory of plant physiology. Plants were grown on hydroponic with half-strength Hoagland`s solution. The following characteristics were measured: number of leaves, length of the green parts of plants, root length, total weight, together with visually assessed plant vigor. Trial included 12 treatments with 30 repetitions each, and that were: leaf extract of comfrey (*Symphitum officinale*), leaf and root extract of nettle (*Urtica dioica*), leaf and root extract of dandelion (*Taraxacum officinale*), extract of above-ground part of horsetail (*Equisetum arvense*), flower extract of yarrow (*Achillea millefolium*), extract of above-ground part of lemon balm (*Melissa officinalis*), root extract of valerian (*Valeriana officinalis*), root extract of burdock (*Arctium lappa*), extract of above-ground part of squirting cucumber (*Ecballium elaterium*), commercially produced gibberellic acid, commercially produced plant growth regulator (PGR) based on seaweed extract and control group.

The results point out the differences in size and direction of impact of various extracts on lettuce`s yield, where some of them behaved even as a retardant. On the basis of this experiment and previous findings, special herbal preparation was composed with the strength of PGR. It`s capacity was tested on lettuce and cabbage in commercial agricultural production with satisfactory results.

Keywords: *herbal extracts, lettuce, yield, PGR*

Introduction

The extracts of wild growing herbs, above all medicinal, are widely used as components of medicinal and cosmetic preparations, as well as various products of the food industry. They are also applied in organic agriculture as mild fertilizers and plant protection means.

A specific type of application of herbal extracts is the production of plant growth regulators (PGR). Here as the raw material most often occur seaweed extracts. The majority of commercially produced extracts derives from brown seaweed, whose representatives are *Ascophyllum nodosum* and species of the genus *Fucus*, *Laminaria*, *Sargassum*, and *Turbinaria* (Calvo et al., 2014). Recently the seaweed extracts are reassigned to the newly formed, broadly structured category - biostimulants. Seaweed used for their production contain auxins, cytokinins and some other hormone-like substances (Bulgari et al., 2015). Beside the extract of seaweed, active dry yeast extracts are in use as well (Hanafy et al., 2012).

Additionally to this raw material base, PGRs can be obtained from extracts of wild growing medicinal herbs. Grounds for such approach are numerous, ranging from the classical authors of Greece and Rome, through Paracelsus, to allelopathy founder Hans Molish and many of his contemporaries, not forgetting biodynamic agriculture practitioners and followers of pure organic gardening.

In ancient times it was already well known that plants contain and release extracts which can produce both positive and negative effects on other plants. The ancient concept of sympathy and antipathy eventually grew into a modern concept of allelopathy (Willis, 2007).

In the Middle Ages, Paracelsus (1525) was among the first to speak about the positive impact of medicinal plants (presumably he was referring to St. John's wort (*Hypericum peforatum*) on the development of other cultures, in the chapter entitled, "About St. John's herbs ".
At the beginning of the 20th century Coupin (1911) studied the effect of herbal ingredients, among them the extract of lemon balm. Molish, founder of the term "allelopathy" demonstrated that flowers and fruits contain many other substances that prove physiological effects on young plants (1937). Ulman (1940) in his doctoral thesis discusses the impact of extracts of yarrow and valerian on the growth of young plants in length. Knapp et Thyssen (1952) undertook a large-scale experiment in which they studied the relationships between 60 species of medicinal plants, grown on experimental plots. The authors have identified three aspects of the plant on the plant impact - 1. stimulating, 2. favorable and 3. adverse effect.
Rudolf Steiner (1993), the father of Anthroposophy and founder of the so-called biodynamic method of agriculture, recommends the following set of species for nutrition and plant protection: - for nutrition: dandelion, English oak bark, nettle, yarrow, valerian;
- for nutrition and protection: comfrey, fern (genus *Telypteris*; syn. *Dryopteris*), great yellow cress, tansy, wormwood. Practitioners of organic farming still use the extracts of elder and rhubarb (Omahen, 1985; Stickland, 1989).
In addition to the above listed, to the present day a large number of plants was recorded whose extracts affect growth, development and the appearance of vegetables and floricultural crops, such as common corn-cockle (Anon., 1986), rape pollen (Thompson, 1981), sage (Ljubojević, 1998) and many others.
Working on the development of special preparations as an alternative for mineral fertilizer to be used in fragile agricultural ecosystems, such as catchment areas of underground drinking water reservoirs, particularly protected area of Vrelo Bosne near the town of Sarajevo, the author conducted a series of experiments aimed at finding the optimal formulation for pre-defined target group of plants. One of the indicative experiments, concerning the testing of different herbal extracts influence on the growth and development of lettuce in early stages, are shown in this paper.

Material and methods

Seed of lettuce (*Lactuca* sativa L.) cv. "Majska kraljica", produced by „Seme", Belgrade (Serbia & Montenegro), were sown at the end of December 1996, at the laboratory of plant physiology in the Agyos Athanasios Industrial Estate, Limassol, Cyprus, on sandy boxes; this leafy vegetable crop showed very high ability in growth during early growth stages (Tei et al., 1996). The bed was regularly watered after sown to ensure proper emergence. When the plants from seed stage entered into cotyledon stage, plants were carefully taken out from sandy boxes and transferred into close hydroponics systems. The nutrient solutions were half-strength Hoagland solution. In the beginning the level of the solution was found to be just above the root hair. After two weeks in the solution the half length of the root was submerged, while the other half was in direct contact with air. The change of the nutrient solution was conducted every four days. Containers with solution were shaken every 12 hours and on every 24 hours fresh air was injected by using the hand pump. In order to provide equal access to light, plants were for 14 hours per day exposed to artificial light which consisted of a couple fluorescent lamps (2 x 60 W) at a distance of 60 cm. Room temperature was maintained at 22 ° C and humidity at 65%.

The moment when the plants from cotyledon stage entered in the seedling stage with the first true leaves, 360 plants were selected and totally randomly distributed in 12 experimental groups with 30 plants in each. In the same way, each group was associated with some of the 11 treatments and one control group. Eight medicinal plants were selected for treatments, which are widespread in our country and a similar climate (Ljubojević et al., 2000, 2014). Considering that the experiment had been carried out on Cyprus, experiment was extended

with a locally widespread plant species, squirting cucumber. Rational basis for this choice lies in the chemical composition of its active constituents and in practice of local rural population to spray fruits and vegetables with a liquid feed diluted with water. Two treatments have the role of rappers. The first one as a strictly specific PGR based on hormonal (gibberellic) effect, and the other as a widely used PGR with various features (seaweed extract). In all treatments and control group, nutrient solution concentration was reduced to 50 % of the standard solution so as to avoid morphological and physiological changes on young plants due to overdose of macro- or microelements.

The treatments were the following:

T1 - Standard nutrient solution (SNS) + water extract of dried comfrey leaf (*Symphyti folia*),
T2 - SNS + water extract of dried nettle leaf and root (*Urticae folium et radix*),
T3 - SNS + water extract of dried dandelion leaf and root (*Taraxaci folium et radix*),
T4 - SNS + water extract of dried aboveground part of horsetail (*Equiseti herba*),
T5 - SNS + water extract of dried aboveground part of yarrow (*Millefolii herba*),
T6 - SNS + water extract of dried lemon balm leaf (*Melissae folium*),
T7 - SNS + water extract of dried valerian root (*Valerianae radix*),
T8 - SNS + water extract of dried burdock root (*Bardanae radix*),
T9 - SNS + water extract of fresh aboveground part of squirting cucumber (*Ecballium elaterium*),
T10 - SNS + water solution of „Giberlex“ – natural plant growth regulator (PGR),
T11 - SNS + water solution of „Original Seaweed Extract“ – natural PGR,
T12 - SNS - half-strength Hoegland solution.

In the foreground of application extracts, all plants were recorded on the following features: the number of true leaves, the length of aboveground parts, root length, total weight. Afterwards four control measurements were carried out during the rosette stage. The experiment was completed before entering the cupping stage, given that the optimal conditions for plants development were not secured in present and subsequent stages of growth.

The impact of treatment on the size of the observed feature is represented by the size of changes with respect to the initial state and their comparison with the control group. The statistical significance of observed differences was evaluated through two-sample t-test, where both samples are small ($n_i \leq 30$) and have equal number of elements. Ranks were assigned using dense ranking.

Preparation and application of treatments

The raw material for preparation of aqueous extracts in treatments T1-T8 consisted of herbal drugs originating from wild medicinal plants, as follows: T1 - comfrey (*Symphitum officinale* L.), T2 - nettle (*Urtica dioica* L.), T3 - dandelion (*Taraxacum officinale* Web. et Wigg.), T4 - horsetail (*Equisetum arvense* L.), T5 - yarrow (*Achillea millefolium* L.), T6 - lemon balm (*Melissa officinalis* L.), T7 - valerian (*Valeriana officinalis* L.), T8 - burdock (*Arctium lappa* L.). The raw material originated from "Herbogal" Svrljig, Serbia, "Krka" d.d., Novo Mesto, Slovenia and "Ulrich" S.p.A, Genoa, Italy. From each plant drug 50 g were taken by and dipped in 450 g of previously softened water, preheated to 65 °C. After 24 hours the mixture was filtered off and stored in a refrigerator. During the application phase the extract concentration in the nutrient solution was 4.76% (1 part of aqueous extract is mixed with 20 parts of nutrient solution).

For the preparation of treatment T9, 150 g of fresh aboveground parts of squirting cucumber was taken (which is equivalent to 50 g of dried herb) and submerged in 450 g of softened water. All other activities remained the same as in the treatment T1-T8. The raw

material was collected in the vicinity of the premises where the laboratory of plant physiology is located (Agios Athanasios Industrial Estate Area, Limassol, Cyprus).

Treatment T10 consisted of the preparation "Giberlex" in the form of tablets, produced by “Fine Agrochemicals Ltd”, UK. One tablet of the preparation contains 1 gram of gibberellins comprising not less than 90% of gibberellic acid and not more than 10% of other biologically active gibberellins. In practice it dissolves with 1,000 litres of water. The same ratio was maintained in the experiment. During application the concentration of previously diluted preparations in the nutrient solution was 4.76% (1 part of pre-diluted mixture was mixed with 20 parts of nutrient solution).

Treatment T11 consisted of the preparation "Liquid Seaweed (Kelp Extract)", derived from fresh Norwegian kelp (*Acrophyllum nodosum* (L.) Le Jolis), produced by “Maxicrop International Ltd.” U.K. One part of mixture was diluted with 400 parts of water. During application the concentration of previously diluted preparations in the nutrient solution was 4.76% (1 part of pre-diluted mixture was mixed with 20 parts of nutrient solution).

The control treatment (T12) consisted of the standard nutrient solution - half-strength Hoagland solution, of the following formulation: N (as NO_3) - 103 ppm, P (as H_3PO_4) - 30 ppm, K - 140 ppm, Ca - 110 ppm, Mg - 24 ppm, S (as SO_4) - 32 ppm, Fe – 2,5 ppm, B – 0,25 ppm, Mn – 0,25 ppm, Zn - 0,025 ppm, Cu – 0,01 ppm, Mo - 0,005 ppm.

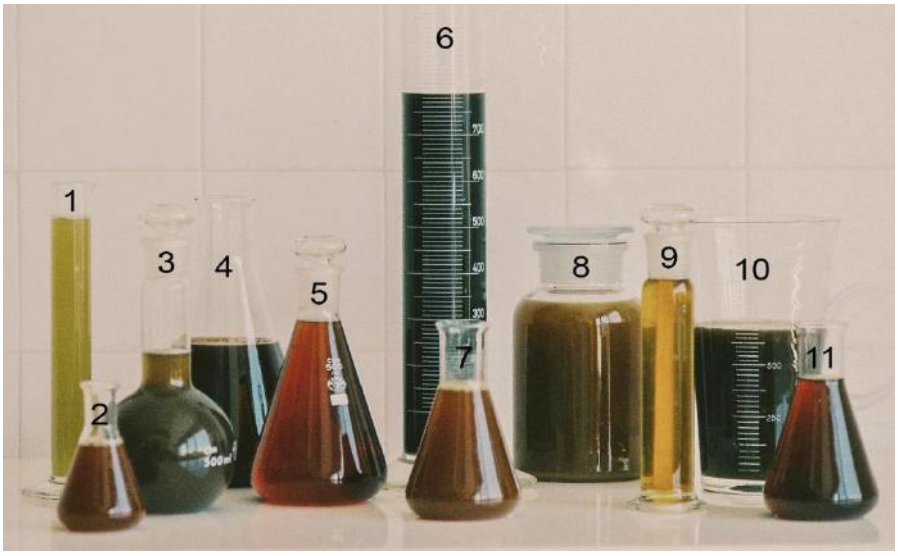

Photo 1: Water extracts used in experiment: 1. of aboveground part of squirting cucumber, 2. of lemon balm leaf, 3. of nettle root, 4. of valerian root, 5. of dandelion root, 6. of aboveground part of yarrow , 7. of nettle leaf, 8. of burdock root, 9. of aboveground part of horsetail, 10. of aboveground part of yarrow, 11. of comfrey leaf

Results and discussion

Number of leaves on the plant

Application of plant extracts did not contribute to a significant increment in the number of leaves on plant compared to the control group. Treatments with comfrey and horsetail led to slowing the development of new leaves, and the differences arising in the reporting period were statistically significant (Table.1). Based on these results we can cautiously conclude that the extracts of comfrey and horsetail act as retardants, when it comes to the number of leaves in the early growth stage of lettuce.

Table 1: The average increase in the number of leaves on the plant

Treatment	Average number of leaves on the plant before treatment	The average increment in the number of leaves on plant during the treatment				Stand. dev. (s)	Test statistic (t_o)	Rank
	Recordings							
	31.12.	22.01.	05.02.	11.02	28.02.			
	(leaves/plant)	(Δ leaves/plant)				(+/-)		
T1 Extract of comfrey	5.4	0.2	1.3	1.8	2.8	0.89	2.84*	9
T2 Extract of nettle	4.3	1.7	1.7	3.7	5.2	2.24		4
T3 Extract of dandelion	4.5	1.3	1.7	2.7	4.9	1.86		6
T4 Extract of horsetail	4.7	2.5	1.7	2.8	1.8	0.74	7.53*	10
T5 Extract of yarrow	4.7	1.6	1.3	2.4	4.8	2.45		7
T6 Extract of lemon balm	4.0	2.2	3.0	2.7	5.0	1.95		5
T7 Extract of valerian	4.0	2.0	2.6	3.6	5.0	2.47		5
T8 Extract of burdock	4.5	2.0	3.7	5.3	4.5	1.92		8
T9 Extr. of squirting cuc.	3.5	2.2	3.2	3.2	5.5	2.15		3
T10 Extract of „Giberlex“	4.7	1.5	2.6	5.3	6.8	3.55		1
T11 Seaweed extract	4.0	1.7	2.3	4.0	5.0	2.95		5
Control group	4.0	1.8	3.8	4.8	5.7	2.76		2

Notice: */ test statistic $t_o > t_1$ (significance level equal to 0.05)

Length of aboveground part of the plant

Application of nettle, yarrow, lemon balm, valerian, burdock and squirting cucumber extracts contributed to increment in length of aboveground part of the plant in comparison to the control group, however, only in the case of lemon balm the observed differences were statistically significant (Tab. 2). On the contrary, with dandelion application the growth of aboveground part of the plant was significantly slowed down. However, the biggest increment in length of above ground part of lettuce has been achieved in treatments with “Giberlex”, while treatments with seaweed extract were lower than the control group.

Table 2: The average increment in length of aboveground part of the plant

Treatment	Average length of aboveground part of the plant before treatment	The average increment in length of aboveground part of the plant during the treatment				Stand. dev. (s)	Test statistic (t_o)	Rank
	Recordings							
	31.12.	22.01.	05.02.	11.02	28.02.			
	(cm/plant)	(Δ cm/plant)				(+/-)		
T1 Extract of comfrey	5.4	1.8	3.3	5.1	13.8	5.73		9
T2 Extract of nettle	5.8	2.9	5.1	12.7	18.7	8.78		3
T3 Extract of dandelion	5.1	4.5	6.2	8.8	9.6	4.8	3.09*	12
T4 Extract of horsetail	5.4	3.4	6.7	9.1	11.9	6.11		11
T5 Extract of yarrow	5.9	2.8	4.1	6.1	18.3	9.46		5
T6 Extract of lemon balm	5.3	6.9	8.2	9.1	21.2	11.6	2.76*	2
T7 Extract of valerian	5.7	4.1	4.9	6.0	18.0	8.42		6
T8 Extract of burdock	5.7	4.2	6.1	8.2	16.5	7.37		7
T9 Extr. of squirting cuc.	4.7	4.6	9.8	14.2	18.5	9.25		4
T10 Extract of „Giberlex“	6.0	4.7	15.7	25.7	33.8	13.6	6.78*	1
T11 Seaweed extract	5.6	2.2	5.1	9.6	13.4	7.91		10
Control group	4.9	4.4	8.1	11.0	14.6	7.45		8

Root length of the plant

Application of nettle, yarrow, valerian and squirting cucumber extracts contributed to increasing the length of roots in comparison to the control group, however, the observed differences were not statistically significant. On the contrary to this, application of comfrey, dandelion and horsetail extracts showed that root growth in length was significantly slowed compared to the control group (Tab. 3). The treatment with lemon balm extract did not affect the root’s growth in length of those intensity as that they did in case of above ground parts. In the case of treatment with "Giberlex" and seaweed extract, root growth trend was slower than the control group.

Table 3: The average increment in length of underground part of the plant

Treatment	Average length of underground part of the plant before treatment	The average increment in length of under ground part of the plant during the treatment				Stand. dev. (s)	Test statistics (t_o)	Rank
	Recordings							
	31.12.	22.01.	05.02.	11.02	28.02.			
	(cm/plant)	(Δ cm/plant)				(+/-)		
T1 Extract of comfrey	3.0	3.0	7.4	8.5	9.7	4.18	3.44*	12
T2 Extract of nettle	2.5	3.7	5.6	12.2	19.0	8.70		1
T3 Extract of dandelion	2.9	2.8	3.4	7.25	10.2	3.98	3.16*	11
T4 Extract of horsetail	3.0	2.1	4.3	10.1	10.7	4.07	2.81*	10
T5 Extract of yarrow	2.6	4.7	5.4	8.8	16.2	7.36		2
T6 Extract of lemon balm	3.7	3.0	4.3	8.9	14.5	7.39		6
T7 Extract of valerian	2.7	2.5	4.0	7.5	16.0	6.80		3
T8 Extract of burdock	3.0	2.5	4.8	9.9	12.7	6.53		7
T9 Extr. of squirting cuc.	3.6	3.4	4.6	13.7	15.2	8.10		4
T10 Extract of „Giberlex“	2.6	5.6	4.7	11.2	12.5	5.46		8
T11 Seaweed extract	2.5	2.8	4.6	7.8	11.5	6.09		9
Control group	2.9	3.4	6.0	12.7	15.0	7.33		5

Fresh weight of the plant

Application of nettle, yarrow, lemon balm and squirting cucumber extracts contributed to the increased growth of fresh plant compared to the control group, however, the observed differences were not statistically significant. On the contrary, treatment with comfrey, dandelion and horsetail gave significantly lower results compared to the control group. From the recordings based on these indicators, we can cautiously conclude that the extracts of comfrey, dandelion and horsetail act as retardants, when it comes to fresh lettuce weight in the early growth stages.

Table 4: The average increment in fresh weight of the plant

Treatment	Average fresh weight of plant before treatment	The average increment in fresh weight of the plant during the treatment				Stand. dev. (s)	Test statistics (t_o)	Rank
	Recordings							
	31.12.	22.01.	05.02.	11.02	28.02.			
	(g/plant)	(Δ g/plant)				(+/-)		
T1 Extract of comfrey	0.54	0.71	1.46	4.03	6.16	2.71	3.98*	12
T2 Extract of nettle	0.63	2.75	3.87	5.14	11.50	6.32		1
T3 Extract of dandelion	0.67	1.23	1.83	4.68	8.50	3.48	2.25*	10
T4 Extract of horsetail	0.60	1.90	2.73	5.65	8.24	3.33	3.35*	11
T5 Extract of yarrow	0.83	2.17	5.17	7.96	11.09	5.66		2
T6 Extract of lemon balm	1.13	3.37	5.62	8.54	10.88	5.40		3
T7 Extract of valerian	0.50	2.33	3.50	5.96	9.58	4.21		7
T8 Extract of burdock	0.83	1.04	2.67	6.77	9.13	4.56		8
T9 Extr. of squirting cuc.	0.80	3.20	3.95	6.36	10.73	5.37		4
T10 Extract of „Giberlex“	1.00	3.50	3.75	8.08	10.52	4.31		5
T11 Seaweed extract	0.83	2.42	3.17	8.92	8.97	5.02		9
Control group	1,00	3.80	7.50	10.12	10.30	5.15		6

Conclusions

The observed plant extracts show contrasting growing patterns in the observed period – at the early growth stages of lettuce. Comfrey extract acts as an inhibitor because it slows down the formation of leaves, development of above ground and underground part in length and conclusive increase of fresh biomass. Similar actions exhibit the extracts of dandelion and horsetail. Nettle extract stimulates the growth of above ground and underground parts of plants, but not to the extent of considering observed changes as statistically significant. An identical conclusion applies to extracts of yarrow, valerian and squirting cucumber. Extract of lemon balm has significant impact on the growth of above ground portions of the plant. Burdock extract does not contribute to a significant increase in above ground and underground parts, but also does not slow down its growth, i.e. does not have the inhibitor characteristics. None of the studied extracts did induce a significant increase in fresh biomass compared to the control group. Nevertheless, the effect of the extract is promising. The majority of extracts has showed without any upgrades the same, if not even better activity than of commercially produced extracts from seaweed. Also, in contrast to the "Giberlex" preparations, whose effect is strictly specific (induces only above ground parts growth), herbal extracts act simultaneously on the above- and underground part of the plant.

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CHEMICAL COMPOSITION AND ANTIOXIDANT POTENTIAL OF ESSENTIAL OIL AND METHANOL EXTRACT FROM MINT (Mentha piperita L.) GROWING IN MACEDONIA

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Abstract

The chemical composition and antioxidant potential of essential oil and methanol extract from Macedonian *Mentha Piperita L.* was object of this study. The plant was organically produced from for the first time at south-east region of Macedonia (41º49`N, 21º 59`E) on the overlapping area of two climate types: the Mediterranean and Continental climate.

The GC-FID and GC-MS analyses of essential oil indicated menthol and menthone as the most abundant compounds with 48.05 and 20.4% respectively. The other identified and quantified compounds such camphene, sabinene, *p*-cymene, *β*-pinene, limonene, *cis*-carvon, menthol acetate, piperitone and piperitone oxide were presented in the levels below 10%.

The total phenolic compounds and total flavonoids were presented at higher level in methanol extract with abundance of 22.42±1.14 mg of gallic acid equivalent/g DM and 0.79±0.34 mg luteolin/g DM respectively, in comparison to essential oil with the levels of 10.12±0.89 mg galiic acid equivalent/g DM and 0.54±0.11 mg luteolin/g DM.

The antioxidant potential measured by two radicals DPPH and ABTS indicated higher values for methanol extract in comparison to essential oil.

Keywords: *Mentha piperita L., essential oil, phenolic compounds, flavonoids, antioxidant potential*

Introduction

The aromatic plant *Mentha piperita L.* belonging to the family Lamiaceae which is grown in regions of Eurasia, South Africa, the north, west and east portions of Europe, as well as Turkey and Russia.

The chemical composition and antimicrobial activity of different varieties of Mentha plant were object of study of many researchers. In the work of Arman et al., the essential oil from *Mentha mozaffarianii* had significant antimicrobial activity against gram positive bacteria *Bacillus subtilis, B. pumulis, Staphylococcus aureus* and *S. Epidermidis* as well as moderate activity against gram negative bacteria such *Escherichia coli*, *Klebsiella pneumoniae*) and no activity against *Pseudomonas aeruginosa, Aspergillus niger*. The major components of the oil were piperitenone (59.5 %), *cis*-piperitenone epoxide (14.9 %), and pulegone (8.5 %), (Arman et al.2011). Chemical composition and antimicrobial activity of *Mentha pulegium* from Iran was studied by Morteza-Semnani et al. (2011). According to their findings, the major constituents of this essential oil were pulegone (54.6 %) and menthone (15.1 %). Carvone-Rich Essential Oils from *Mentha longifolia (L.) Huds. ssp. schimperi Briq*. and *Mentha spicata L.* grown in Sudan had oxygenated monoterpenes comprised 81.5% and 88.7%, while monoterpene hydrocarbons comprised 14.7% and 9.2% (M. H. Younis and S. M. Beshir 2011). Zeinali et al. (2005), reported fifteen principal components in the oils of 12 variety of Iranian mints accessions. The oils obtained from Mint variety Mzin 9 and Mzin 10 contained the highest value of *p*-cymene with the levels of 48.9 and 48.6%. In the mint oils from variety Mzin 5 and Mzin 11 was quantified cis-carveol over 70%. Carvon oxide was the most dominant compound in Mzin 4 with the level of 52.5%. Rissanen et al. (2002), stated that oil

yield and composition of *Mentha piperita L*. depends of plant density and growing season. According to their findings, the highest oil concentrations were present in highest plant densities in the first growing season.

The chemical composition and antioxidant potential of essential oil and methanol extract from Macedonian organically produced *Mentha Piperita L.* was object of this study.

The plant was organically produced, described in details in research of Mihajlov et al. (2015), from for the first time at south-east region of Macedonia (41º49`N, 21º 59`E), on the overlapping area of two climate types: the Mediterranean and Continental climate.

Materials and Methods

Oil isolation and analysis: A sample of 250 g of dried leaves and stems was mixed with 500 mL of tap water in flask and water distilled for 2 h using a Clevenger-type apparatus (Fig. 1). The oil content was measured based on mL oil per 100 g dry matter (mL/DM). The oil compositions were analyzed by GC (FID) and GC/MS. Gas chromatography was carried out with an Agilent HP 6890 gas chromatograph equipped with flame-ionization detector (FID) and quantitation was carried out by addition of pure standards as well as area normalization and neglecting response factors.

The analysis was conducted using a HP-5 (5% Phenyl Methyl Siloxane) fused silica capillary column (30 m x 0.50 mm, film thickness 0.32 μm, J &W Scientific Inc., Rancho Cordova, CA). The operating conditions were as follow: injector and detector temperature: 250°C, carrier gas: helium; inlet pressure: 35.4 kPa. Oven temperature program was 50 - 220°C at the rate of 4°C/min. Quantitative data concerning the percentage contribution of each constituent were taken with this system. GC/MS analysis was carried out using an Agilent HP 6890 gas chromatograph fitted with the same column as described above, coupled to quadrupole 5973 MSD, which was operated at an ionization potential of 70 eV and electron multiplier energy 2000 V. The temperate program started at 50°C during the split injection and then programmed to 220°C with increment of 4°C/min. The oil components were identified by comparing their retention indices and mass spectra data (NIST 14 Standard Reference Database 1A) with those of authentic samples and published data: M. H. Younis and S. M. Beshir (2011), S. Kostadinović Veličkovska (2013), and S. Kostadinović et al. (2010).

Determination of total phenolic compounds and total flavonoids: 0.5 mL of the methanol extract and 0.5 mL of essential oil were dissolved in 5 mL distilled water and mixed with 0.5 mL of 10 times diluted Folin-Ciocalteu’s reagent. 1 mL of saturated sodium carbonate (35 %) was added to the mixture and it was topped up to 10 mL distillated water. After three hours, the total phenolics were measured spectrophotometrically at 725 nm.

Determination of flavonoids: in the extract and essential oil was performed by method of Oomah et al, (1996). Methanolic extract and essential oil (10 μL) was three times dissolved in distillated water. Furthermore, 200 μL of diphenylboric acid 2-aminoethyl ester solution was added in the mixture and solution was measured at 404 nm. Luteolin as standard for calibration curve was used in the range from 0.1 to 10 mg/L.

Antioxidant assays: DPPH and ABTS: For DPPH assay, the antioxidant activities of the extract and essential oil were expressed as percentage of decolorization of a solution of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical) at 517 nm.The Trolox equivalent antioxidant assay (TEAC) employed in this study gives a measure of the antioxidant activity of methanol extract and essential oil under study. For this purpose 10 mL of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) solution was prepared from 39.23 mg of ABTS and 7.17 mg of potassium thiosulphate disolved in Nanopure water to volume.

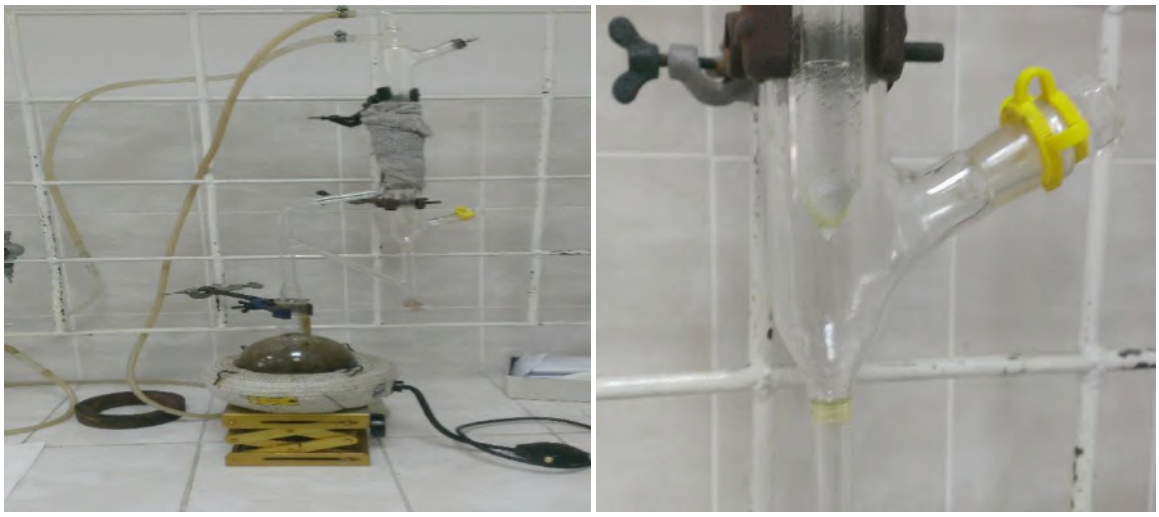

Fig. 1. Isolation of essential oil from *Mentha piperita L.* by hydrodestilation.

Results and Discussion

The qualitative and quantitative compositions of the essential oil obtained from Macedonian organically produced *Mentha piperita L.* was presented in Table 1. Twelve components were identified with total abundance of 85.96%. Four compounds had the highest abundance from all identified compounds, in particular: menthol (48.05%), menthone (20.4%), piperitone (8.3%) and piperitone oxide (4.9%). The relative percentages of all other compounds were in the level below 2.5%. The level of total phenolic compounds and total flavonoids for methanol extract and essential oil of organically produced *Mentha pipperita L.* as well as antioxidant activity were presented in Table 2. As we can see from the results, the total phenolic compounds expressed as gallic acid equivalent had higher abundance in comparison to total flavonoids expressed as mg of luteolin/g of dry matter.

On the other hand, menthanol extract had higher level of both classes of polyphenolics in comparison to the level of the same classes of polyphenolics in distillated essential oil. Furthermore, the level of total phenolic compounds for mentanol extract was more than double in comparison to total phenolics found in essential oil. However the difference for flavonoids was around 30% in favor to methanol extract. The higher antioxidant potential by two radicals (ABTS and DPPH) had methanol extract. More precisely, methanol extract had higher antioxidant potential than essential oil which was in good agreement with the results from total phenolics and total flavonoids. On the other hand, TEAC assay showed higher antioxidant potential for both samples in comparison to DPPH assay. If we compare the results from DPPH and ABTS assay for methanol extract and essential oil presented, we can concluded that results are more than double in favor to ABTS assay.

Table 1. Chemical composition of essential oil from *Mentha piperita L.* from Macedonia by GC-FID and GC-MS

	Compound	RT	Area (%)
1.	camphene	947	0.05
2.	sabinene	974	0.10
3	*p*-cimene	1025	0.05
4.	β-pinene	1027	0.01
5.	limonene	1034	1.2
7.	**menthone**	1110	**20.4**
8.	**menthol**	1175	**48.05**
9.	*cis*-carveol	1180	2.2
10.	menthol acetate	1183	0.7
11.	piperitone	1185	**8.3**
12.	piperitone oxide	1199	**4.9**
	Total		**85.96**

Table 2. Total phenolic compounds, total flavonoids and anthioxidant activity of methanol extract and essential oil from Mentha piperita L.

	Total phenolic compounds (mg GAE/g DM)	Total flavonoids (mg Luteolin/g DM)
Methanol extract of *Mentha piperita L.*	**22.42±1.14**	**0.79±0.34**
Essential oil from *Mentha piperita L.*	10.12±0.89	0.54±0.11
	DPPH assay (mg Trolox/g DM)	TEAC assay (mg Trolox/ g DM)
Methanol extract of *Mentha piperita L.*	12.98±1.05	**27.14±2.24**
Essential oil from *Mentha piperita L.*	7.14±0.87	**13.31±0.04**

Conclusion

We concluded that the region of South-east Macedonia had good potential for production of high-quality organically produced *Mint (Mentha piperita L.)* described in details in published surveys of Mihajlov et al. (2015), with appreciable amount of menthol and menthone.
Further investigations will include antibacterial, antifungal and antimicrobial activity of essential oil from different varieties of Mentha plants.

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ECONOMIC CHALLENGES OF ORGANIC FARMS IN POLAND

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Abstract

Agricultural holdings are facing many challenges, which they must meet. These challenges are conditioned, on the one hand by macroeconomic factors, such as progressing globalisation and growing consumers awareness concerning the quality of agricultural products, as well as the need to respect natural environment. On the other hand, farms as enterprises must be ruled by the microeconomic conditions, namely the agricultural production volume and value, as well as satisfactory level of agricultural income. There is common understanding, that only conventional farms can cope with the microeconomic and global challenges.

The purpose of article is verifying, basing on the microeconomic account, if organic farms are able to meet the economic challenges. The article focuses on the analyses of production and economic results of organic farms compared to all private farm covered by agricultural accountancy system in Poland – Farm Accountancy Data Network (FADN) 2012. The study was conducted taking into consideration agricultural land and farming types that indicate specialization of agricultural production. There were calculated the main indicators, namely: land and labour productivity, as well as profitability, using the following categories: total agricultural output, gross farm income, agricultural farm income.

Conducted research in static approach indicated, that organic farms have smaller ability to meet the economic challenges than conventional ones, however, in the long term and with full economic account including the externalities, these relations would probably be opposite in favour of sustainable form of agriculture. On the basis of the relatively unfavourable economic results of organic farms, it can be concluded that the financial support under governmental programmes targeting such a system of farming is inadequate.

Keywords: *Organic farms, microeconomic account, Poland, FADN data*

Introduction

The increasing liberalisation of markets in terms of structural supply surpluses puts competitiveness on a pedestal, meeting of which is essential for the achievement of the economic objectives of agricultural holdings. Competitiveness cannot be achieved at the expense of the natural environment or the food quality. These requirements try to reconcile the organic form of agriculture, which becomes a part of the concept of sustainable agriculture. The key feature of organic agriculture is the use of such methods of agricultural production, which comply with the requirements of soil fertility, plants and animals, with simultaneous care of the other environmental components (Nachtman, 2015; Runowski, 2012; Sołtysiak, 1994).

The development of organic farms is dependent on economic relations and national policy (Wrzaszcz and Zegar, 2014a; Wrzaszcz and Zegar, 2014). Organic production, on the one hand, should not affect the ecosystem sustainability, on the other hand, must meet economic challenges. Meeting these challenges may occur in the way of normal market rules – *via* higher prices and possibly lower costs of production – or (and) targeted support of governmental institutions, primarily in the form of subsidies. The first one is observed for example in American agriculture, while the other in the agriculture of the European Union countries, including Poland.

This article aims at verifying, basing on the microeconomic account, if organic farms are able to meet the economic challenges.

Material and methods

The analysis considered 10,589 farms covered by the Farm Accountancy Data Network in Poland in 2012. The article focuses on the production and economic results of organic farms compared to all individual (private) farms covered by FADN. The guiding principle in the organic system is to cultivate plants in compliance with the standards of the *Good agrienvironmental practices* with due attention to the phytosanitary condition of plants and soil protection. Such farms are obliged to keep an area of permanent grasslands and landscape elements not used for agricultural purposes[8].

For analysed groups of farms, there was presented their number and the basic characteristics of the production potential, measured by the following indicators: the area of agricultural land (AL ha), the labour input (AWU[9]), the stocking density (LU[10]) and the standard gross margin (ESU[11]). The study was conducted taking into consideration farming types that indicate specialization of production, namely: specialised in field crops (type: I), in permanent crops (III) and in rearing grazing livestock (IV), non-specialised with mixed crops (VI), with mixed livestock (VII) and with mixed crops and livestock (VIII).

Subsequently, the economic efficiency of farms was assessed by means of the following indicators: land productivity (Total Output/Agricultural Land; Gross Farm Income/Agricultural Land); labour productivity (Total Output/Annual Work Unit; Gross Farm Income/Annual Work Unit); land profitability (Family Farm Income/Agricultural Land); labour profitability (Family Farm Income/Family Work Unit). *The Total Output* of a farm indicates the economic result of farming. It is the outcome of the sum of the crop and livestock production value and other activities. *Gross Farm Income* is the result of difference of total output and the total intermediate consumption (total specific costs – including inputs produced on the holding – and overheads arising from production in the accounting year), adjusted for the outcome of the balance of current subsidies and taxes. It accounts the impact of the agricultural policy on the economic situation of farms by means of the system of subsidies and taxes (Goraj, 2009). Net value added adjusted for the cost of total external factors and the balance of subsidies and taxes on investments indicates *the level of Family Farm Income* (Bocian and Malanowska, 2014). *Family Farm Income* is the primary economic goal of farmer's agricultural activity and it is an essential determinant of a farmer family living standard, and hence it may be an important indicator of farm efficiency in agriculture. The issue of factor productivity is especially significant in the context of food security, thus profitability factor has particular importance in the evaluation of labour remuneration and farmer`s family general income. The volume of income illustrates the level of compensation for the involvement of their own factors of production in the farm's operations and for the risk taken by a farm manager in a given financial year.

[8] The list of legal regulations in the field of organic farming system can be found on the website of the Ministry of Agriculture and Rural Development: (http://www.minrol.gov.pl).

[9] 1 AWU (Annual Work Unit) is equivalent to full-time own and paid labour, i.e. 2,120 hours of work a year. 1 FWU (Family Work Unit) is the equivalent of a full-time labour of a farming family member.

[10] 1 LU – Livestock Unit is a conventional unit of farm animals with a mass of 500 kg.

[11] Sum of standard gross margins – the difference between output and specific (direct) costs of all activities occurring on the farm – indicates the economic size of the farm, otherwise the productive potential of the farm. 1 ESU is equivalent to EUR 1,200. The standard gross margin is the average gross margin by region. Standard gross margin on a particular crop or animal is a standard (average of three years in a particular region) value of production obtained from one hectare or from one animal minus the standard direct costs necessary for production.

Results and discussion

Organic farms constitute a small fraction – 4% of FADN farms` population (Table 1). The average area of the organic farms amounted to 37 ha, just like the size of an average individual one. Only 40% of organic farms had an area of at least 50 ha, whereas every second farm had such an area (Chart 1a). The level of labour inputs was slightly lower in the case of organic farms. The average size of a herd of animals in the individual farm in question amounted to 27 LU. In comparison to that, the organic farms significantly deviated from the average (14 LU). Every fifth individual farm didn't conduct animal production, whereas among organic ones it was every fourth. Such a result is not justified for organic farms. The main idea of organic production is to keep a closed circuit of nutrients within a farm, which should lead to mixed agricultural production. The presented data confirm the discrepancy between theory and practice in agriculture[12]. The differences in the livestock population of the selected groups of farms were reflected in the total value of their assets. Organic farms were characterised by the lowest value of the assets (ca. ¼ as compared to an average individual farm). The standard gross margin made it possible to synthetically evaluate the economic potential of the identified groups of farms. Organic farms differ in this respect from the average individual farm – the result was lower by 38%. This result is a proof of the importance of animal production and diverse crop production for the value of the standard gross margin. Multifunctionality of agricultural production and diversified crop structure contributes not only to the assurance of environmental benefits, but also tangible economic benefits.

Table 1. Production potential of analysed farms` groups (average per farm)

No.	Specification	Total farms	Organic farms (ECO)	ECO/Total (%)
1	Number of farms	10 589	422	3,9
2	Agricultural land (ha)	36,8	37,4	101,5
3	Labour input (AWU)	2,01	1,89	94,0
4	Livestock units (LU)	27,3	14,4	52,6
5	Total assets (thousand EUR)	294,3	214,5	72,9
6	Standard gross margin (ESU)	21,7	13,5	62,2

Source: Prepared on the basis of FADN 2012 data.

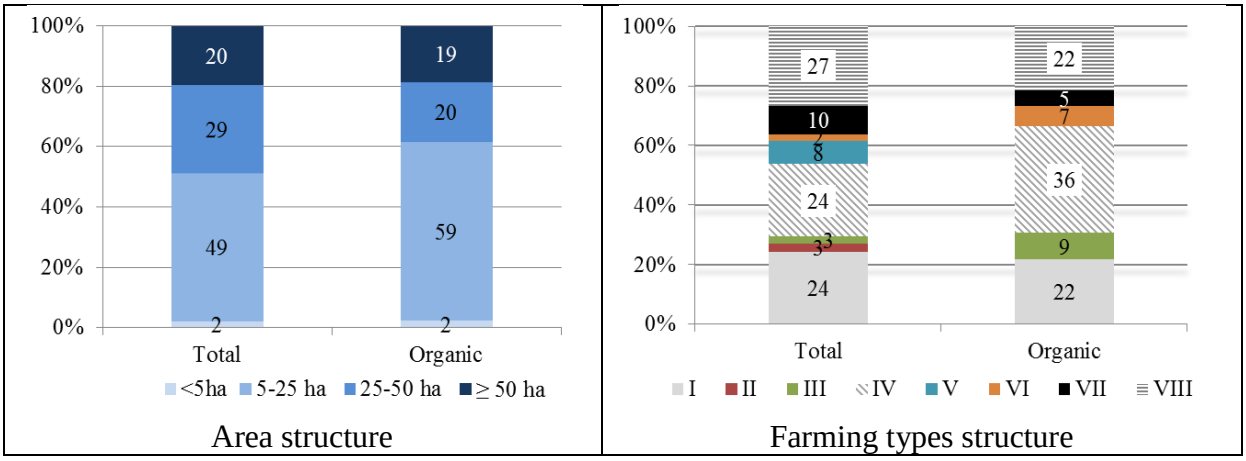
About 60% of the analysed farms` population are specialized holdings – mainly focused on field crops (I) and rearing grazing livestock (IV, Chart 1b). Among the non-specialized farms – with the so-called mixed-production – dominate the holdings, with mixed crops and livestock (VIII). However, organic farms are more likely to lead a specialized agricultural production.

The value of agricultural production is predominantly determined by the total output value. The relatively largest importance of the other kind of production can be seen in organic farms (3% of total output value), which results from slightly different organisation of such farms. In the case of many organic farms, agricultural production is supplemented for instance with agri-tourism based on the assets of a farm. Land productivity on an average individual farm amounted to EUR 1,691/ha[13] (Table 2). Compared to that, organic farms deviated from the average individual one in particular – their result was lower by 58%. The presented figures confirm lower land productivity on farms that provide services for the natural environment – of course if we measure it in static terms. It is very likely that in the long term and with a full

[12] Studies have demonstrated, that there is a growing problem involving not only limiting the scale of animal production on organic farms, but even giving up such production (Nachtman, 2014; Wrzaszcz and Zegar, 2014a).

[13] The exchange rate in FADN 2012: EUR 1 = PLN 4,3177.

account of the costs and benefits – of the externalities – these relations would be the opposite. Organic farms – just like in terms of land productivity – had significantly lower results in **labour productivity** (with the difference amounting to 54%).



Area structure — Farming types structure

Chart 1. Area (a) and farming type (b) structure of all and organic farms

Source: Prepared on the basis of FADN 2012 data.

Table 2. Selected categories of economic calculation for analysed total and organic farms

No.	Specification	Total farms	Organic farms (ECO)	ECO/Total (%)
1	**Total Output (€/ha AL)**	**1691**	**714**	**42,2**
2	*Total Output (€/AWU)*	*30 945*	*14 160*	*45,8*
3	Total intermediate consumption (€/ha AL)	1 003	407	40,5
4	Balance on current subsidies & taxes (€/ha AL)	216	319	147,9
5	Agri-environmental subsidies (€/ha AL)	22	113	504,6
	Organic subsidies (€/ha AL)	4	101	2543,9
6	**Gross Farm Income (€/ha AL)**	**904**	**627**	**69,4**
7	*Gross Farm Income (€/AWU)*	*16 540*	*12 423*	*75,1*
8	Depreciation (€/ha AL)	205	140	68,5
9	**Farm Net Value Added (€/ha AL)**	**699**	**487**	**69,7**
10	Total external factors*	78	70	90,1
11	Balance on subsidies & taxes on investments	-40	-19	47,1
12	**Family Farm Income (€/ha AL)**	**633**	**425**	**67,1**
13	*Family Farm Income (€/FWU)*	*13 363*	*10 334*	*77,3*

* The salaries of employees (including social insurance of salaried employees), the rents associated with the lease of land and buildings and the interest and fees associated with the loans contracted for the purchase of land, buildings, machinery, equipment, livestock and materials.

Source: Prepared on the basis of FADN 2012 data.

Total intermediate consumption on an average individual farm amounted to EUR 1,003/ha (Table 2). In this respect, farms that provide services for the natural environment were characterised by a lower production intensity. Organic farms distinguished themselves in this respect in particular, which results from the specifics of this production system that is based on small and justified use of external means of production.

The Common Agricultural Policy instruments that are currently under implementation determine the opportunities for obtaining financial support by agricultural producers through environmental restrictions and for compensating the services provided for the environment. These determinants have the form of regulations, such as cross-compliance, that are linked to

direct payments, refer to diversification of that type of payments depending on the chosen plant species and animals production and include the activities for the sustainable development in the form of e.g. environmental programmes[14]. The implementation of these measures by farmers is confirmed by the level of subsidies they receive. An account drawn up in accordance with the FADN methodology distinguishes the category of the balance of subsidies and taxes connected with current productive activity. The category indicates the direction of the transfer of funds to a farm when it adopts a positive value. For organic farms, this category was significantly higher than the average result for the population of the analysed farms (48%). About one third of current subsidies are organic subsidies, that accounted 90% of agri-environmental payments of organic farms. These differences indicate that the measures taken for the environment are compensated by the state with funds, however there is still a question whether they are sufficient.

Gross farm income on an average individual farm amounted to EUR 904/ha. The conducted static analysis indicates that organic farms are incomparably lower than the other farms in terms of produced volume of agricultural production (31%), and policy instruments compensate for such differences only to a certain degree. Despite a high share of the balance value of current subsidies and taxes in the gross farm income in the case of organic farms (51%), their results are still below an average one.

Land and labour profitability in family farms are illustrated in table 2. On average, the use of 1 ha of utilised agricultural area made it possible to generate income amounting to EUR 633/ha. Organic farms still have a significant *economic delay* compare to other analysed farms (EUR 425/ha, the difference 33%). The presented results may be a sign of insufficient *legal and financial protection* of that production system because they compensate for a small part of the low profitability of the production factors. One full-time worker of family labour on an average individual farm (that indicate labour profitability) achieved the result of EUR 13,363/FWU. Unfortunately, organic farms are still placed on unsatisfactory position (EUR 10,334/FWU, the negative difference amounting to 23% compared to the average FADN farm), which strengthens previous conclusions on this system of farming.

In the analysed population, farms specialized in permanent crops (III) characterised with the highest **land productivity and profitability**, while on the opposite place, holdings specialized in field crops are located (I, Chart 2a). Regarding organic farms, specialized ones generate less satisfactory production and economic results than non-specialised (Chart 2b). This result is promising in the light of sustainable development – diverse organic production not only provides relatively greater environmental benefits, but also economical ones. However, organic farms remain far behind average individual holdings in terms of land productivity and profitability (the difference in the case of total output, gross farm income and family farm income per hectare of AL, was appropriately 58%, 31% and 33%).

Farms with mixed livestock (VII) characterize with relatively less surface of agricultural land, resulting in a higher production outcome per hectare. While the level of unit costs (intermediate consumption measured per unit area) is nearly twice higher in those farms compared to entities specialised in field crops (I), simultaneously having lower subsidies to current activity. The level of subsidies, connected with, among other, with providing environmental services as a part of the Rural Development Programme, was a significant factor determining the higher profitability of farms specialized in field crops (I).

Organic farms – like in terms of land productivity and profitability – significantly deviate from all individual farms *in minus* in the range of **the labour productivity and profitability** (the difference in the case of total output, gross farm income and family farm income per work unit, amounts appropriately to 54%, 25% and 23%, Chart 2c,d). Regarding the farming

[14] www.minrol.gov.pl.

type, agricultural holdings specialized in field crops (I) achieve the best results in this range, in contrast to those with mixed crop production (VI). This relationship is maintained also in the case of organic farms. In the case of organic farms, the attention is put towards agricultural holdings specialized in permanent crops (III), where in spite of relatively lower unit value of total output per annual work unit, unit gross farm income and family farm income exceed the average result for all organic farms. The national system of agri-environmental subsidies particularly prefers this farming type, that is reflected in the presented economic account (Table 3).

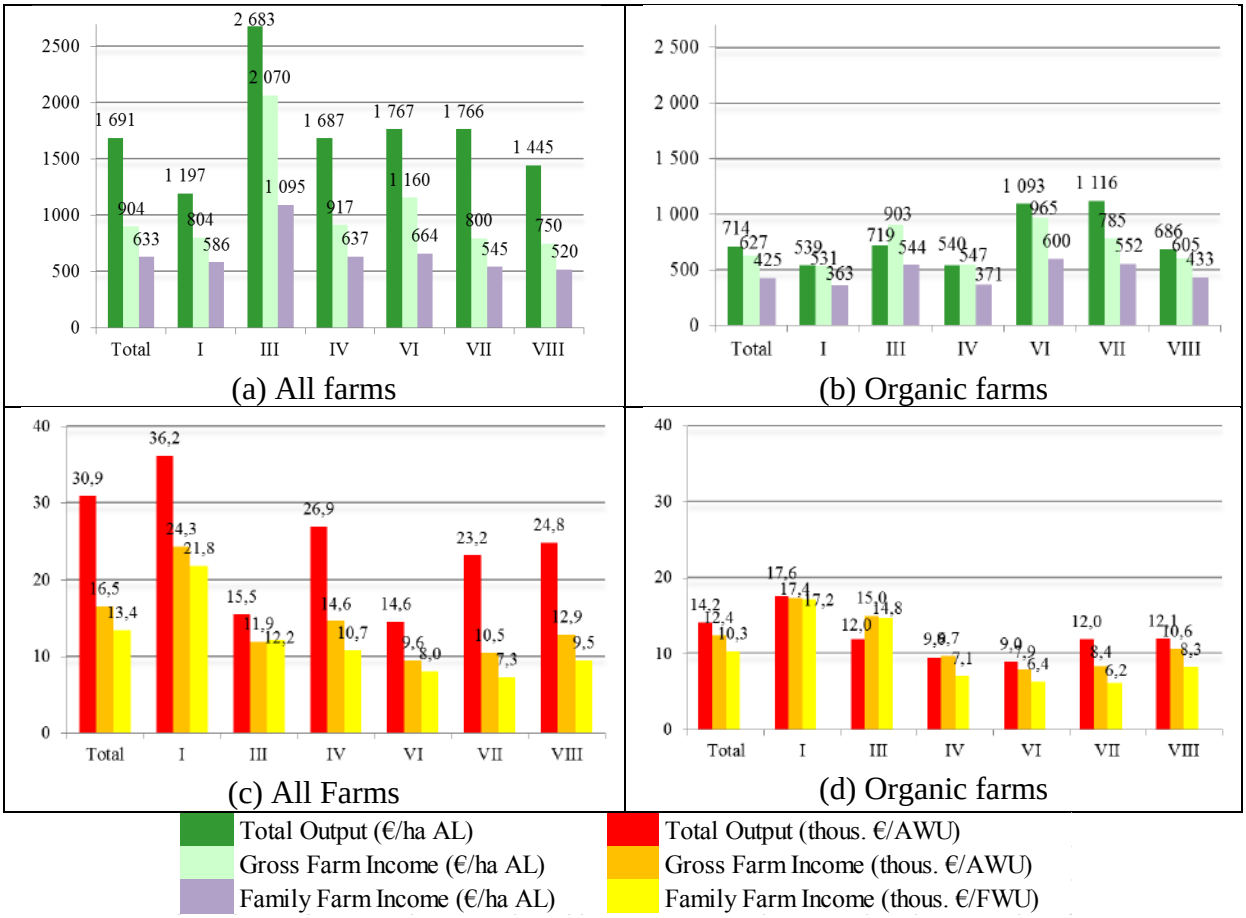


Chart 2a-d. Land (a, b) and labour (c, d) productivity and profitability of all and organic farms
Source: Prepared on the basis of FADN 2012 data.

Table 3. The level of agri-environmental subsidies in total and organic farms

Subsidies	Total	I	III	IV	VI	VII	VIII
Agri-environmental subsidies (€/ha AL)	22,3	25,4	57,9	15,5	26,9	18,2	24,0
Organic subsidies (€/ha AL)	4,0	3,6	56,6	4,3	11,8	1,3	2,4
Agri-environmental subsidies/Family Farm Income (%)	3,5	4,3	5,3	2,4	4,0	3,3	4,6
Organic subsidies/Family Farm Income (%)	0,6	0,6	5,2	0,7	1,8	0,2	0,5
Subsidies	**Organic**	**I**	**III**	**IV**	**VI**	**VII**	**VIII**
Agri-environmental subsidies (€/ha AL)	112,6	96,0	258,3	107,5	139,0	98,0	89,1
Organic subsidies (€/ha AL)	100,7	91,7	255,1	83,8	132,3	88,5	80,5
Agri-environmental subsidies/Family Farm Income (%)	26,5	26,5	47,5	29,0	23,2	17,8	20,6
Organic subsidies/Family Farm Income (%)	23,7	25,3	46,9	22,6	22,0	16,0	18,6

Source: Prepared on the basis of FADN 2012 data.

Conclusion

The number of organic farms is not numerous. Production and economic results of organic farms are significantly lower than for the average individual farm, both in terms of livestock population, assets and the generated standard gross margin. This result is a proof of the importance of animal production and diverse agricultural production for the value of the standard gross margin.

Organic farms do not match the other farms in terms of the generated production volume and land productivity.

Organic farms could not match the average ones in the range of land and labour profitability. Unfortunately, also in this regard, organic farms generate worse economic effects. On the basis of the relatively unfavourable economic results of organic farms, it can be concluded that the financial support under governmental programmes targeting such a system of farming is inadequate.

Regardless the farming type, economic efficiency of organic farms is lower compared to the average individual farms.

Regarding the farming type, in particular, organic farms specialised in field crops (I) and in rearing grazing livestock (IV) generate significantly lower results in terms of land productivity and profitability in relation to average individual farms of mentioned types.

Conducted research in static approach indicates, that organic farms have smaller ability to meet the economic challenges than conventional ones, however, in the long term and with full economic account including the externalities, these relations would probably be opposite in favour of sustainable form of agriculture.

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THE POSSIBILITY OF USING WASTE BIOGAS PLANT (DRY RESIDUE AND PELLETS) AS FERTILIZER

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Abstract

Increased requirements to protect the environment from pollution, ever increasing cost of waste rekindled interest in the anaerobic digestion of biomass in biogas plants. Fermented sludge from biomass is a valuable fertilizer, since the fermentation process is stored in the organic or ammonium form almost all of the nitrogen. It does not contain pathogenic microflora and viable weed seeds, suitable for aerobic composting or direct use as a fertilizer in the form of solids or pellets. The purpose of the study - to explore the possibility of using waste biogas plant as fertilizer in a laboratory experiment. The most effective stimulation of the fertilizer for growth and weight of aboveground parts of barley are pellets at a dose of 2.20 g / kg 4.28 g / kg and a dry residue at a dose of 1.36 g / kg. The least effective option turned making pellets at a dose of 1.12 g / kg. This is because the sawdust pellets contained in conifers has a high ratio of carbon to nitrogen, for supplying negative microorganisms which does not compensate for the nitrogen contained in the pellets in a minimum dose. Making pellets and solids at all doses increased cellulolytic activity to 79-91 % in the case of pellets (with increasing doses reduced activity) to 82-90 % in the case of the dry residue (increasing doses led to an increase in activity). Most cellulolytic activity observed when making pellets at a dose of 1.12 g / kg solids in a dose of 5.42 g / kg.

Keywords: *biogas, pellets, organic fertilizer, barley, laboratory experience.*

Introduction

Annually Russianlivestock farms produce 200-250 million tons of manure and about 600 million tons of liquid effluents. Organic waste without recycling can significantly pollute the environment. The chemical composition of manure is a valuable secondary resource. Therefore the main objective of its use is the use as organic fertilizer on agricultural land.

The first of the ecological problems of industrial livestock production is the transformation of potentially hazardous environmental contaminant – manure, relatively strong, fast, and complete organic fertilizer, rich in nutrients. You need to look for new ways of processing that would meet the following requirements: disinfection, ensuring long-term storage with maximum retention of nutrients, elimination of waste, polluting the environment. At the same time, it is important that they are economically justified. For the processing of manure can be applied biotechnology and by regulating the activity of microorganisms, to obtain high quality products, while preserving the environment (Orlova, 2009).

Increasing requirements to protect the environment from pollution, increasing the cost of recycling has revived interest in the anaerobic digestion of biomass in biogas plants. The biogas plant not only lower the hazard class of livestock waste and contribute to production of organic fertilizers, which are due to the anaerobic treatment is easily absorbed by plants, but also allow to obtain electrical and thermal energy (Lysenko, 1998). The fermented material slurry after processing of biomass is a valuable fertilizer, as in the fermentation process and stored in the organic or ammoniums form almost all of the nitrogen. It does not contain pathogenic microorganisms and viable weed seeds suitable for aerobic composting or direct use as fertilizer in the form of solids or pellets.

The aim of the study was to explore the possibility of using waste biogas plants as fertilizer laboratory experience.
In accordance with the aim, the following tasks were set:
1. Agrochemical analysisof soilis making for laboratorial test.
2. Influence assessment of different forms and doses of fertilizers from cattle manure in comparison with urea on growth of the aerial parts of barley.
3. Influence assessment of different forms and doses of fertilizers on biometrics of barley.
4. Influence assessment of different forms and doses of fertilizers on the effect size of phytostimulation.
5. Influence assessment of different forms and doses of fertilizers on the cellulolytic activity of the soil.

Materials and methods

The objects of study are the dry residue and pellets.The dry residueisobtainedby separation from cattle manure. Pellets are made by line LCC-500 from the enterprise "Integrated recycling system" the city of Orenburg (Russia). Line for the production of fertilizers in the form of pellets required for the production of fuel pellets from the solid fraction of the biogas plant. These pellets can be used as fuel for heating. The heat transfer is several times higher than that of wood of wood for 1 kg of fuel: allocated 4400 kcal of heat energy. However, it is worth noting that the granules of litter and manure are almost unusable to pellet boilers. They long to ignite, has a bad smell and the combustion temperature is ineffective. Nevertheless, the experimenter said, “It is best to use granular manure as fertilizer, not for burning”. The company "Energorezhim" (the city of Perm (Russia)) on the enterprise "Integrated recycling system" the city of Orenburg (Russia) in 2012 launched a line of pellet production from cattle manure. This product can be used in dry form as a soil fertilizer in recommended doses. The same can be soaked in water. This gives an aqueous solution of an organic fertilizer with all necessary nutrients.
According to research conducted by the Testing laboratory FGPAS "Orenburg" the content of basic nutrients in dry matter: organic matter is 57.5%, total nitrogen – 5.53 %, total phosphorus – by 1.4 %, total potassium was 8.8 %, nitrate nitrogen – 10388 mg/kg ammonium nitrogen – 82946 mg/kg, movable copper – 3.6 mg/kg, movable zinc – 17.4 mg/kg, movable iron to 78.3 mg/kg.
The content of basic nutrients in the pellets: total nitrogen – 6.7%, total phosphorus – 1.1%, total potassium of 7.0%, sawdust coniferous trees – 20.0% by weight.
For comparison we used nitrogen fertilizer urea $CO(NH_2)_2$ with a nitrogen – 46.2%.
The experiments were performed in the chemical laboratory of ecology Department of the Perm state agricultural Academy in the 2012-13 year. Soil for laboratory experiences taken in Krestyanskih – farm SP Raszyn. Soil is sod-podzolic heavy loam.
Agrochemical analysis was performed according to standard procedures. Determination of phytotoxicity was carried out by means of seedlings. The method is based on the reaction of the test culture, when applied to the soil or other compounds, which reveals the toxic effect or a stimulating effect of introduced substances. As the test object was used germinated seeds of barley varieties Rodnik Prikamya. Calculation of doses of fertilizers produced on the active substance (nitrogen) per 1 kg of soil.
Options: without fertilizer (control), urea 0.16 g/kg, urea 0.32 g/kg, urea 0.64 g/kg, dry residue of 1.36 g/kg, residue is 2.71 g/kg dry residue 5.42 g/kg, pellets of 1.12 g/kg, pellets 2.20 g/kg, pellets to 4.28 g/kg.
The experiment was conducted in triplicates. In a plastic container with a volume of 500 cm^3 was placed 400 g of the prepared substrate is pre-wetted to 60% of moisture capacity. In each vessel planted 15 seeds of the studied culture. On the fourth day the container was placed on a

rack with lighting within 24 hours. In the course of the experiment was maintained constant humidity. Length of experience is 14 days. When experiment plants were cleared from the ground and dried. Then experimenters determined the height of seedlings of barley, root length, weight of aerial parts and roots (Orlov, 2002).

The effect of phytostimulation (FS) was calculated according to the weight of aerial parts and roots of the following equation:

$$FS, \% = (M_H - M_K)/M_X \text{ ,where}$$

M_K is the mass of plants in the control vessel, g;

M_X is the mass in the vessel of the investigated variants, g.

To determine cellulolytic activity of the soil in the Petri dishes were taken 100 g of soil, with subsequent irrigation of up to 80% of full soil water content. 10 pieces of linen fabric 1x1 cm evenly placed in cups with soil. After that, the cups were covered with lids and left for 30 days at room temperature. The experiment was conducted in four replications. Options experience is similar to previous experience.

Results and discussion

To conduct the study and determine the optimal doses of fertilizer selected sod-podzolic heavy loam soil in farm SP Raszyn, which is located in the village of the Katishi of Krasnokamsk district of Perm Krai. Next table shows results of agrochemical analysis of soil.

Table - Agrochemical indicators of sod-podzolic soil

Humus, %	pH_{KCl}	Hr, mEq/100 g	S, mEq/100 g	PC, mEq/100 g	V,%	$N_{tot.}$, %	$P_2O_{5mob.}$, mg/kg
5.4±0.6	6.2±0.2	1.1±0.2	23.4±1.3	24.5	95.6	0.37±0.03	198±62

Humus contributes to the formation of stable soil structure, provides a favorable water-air regime, gives the soil buffering in relation to nutrients, especially nitrogen. High level of microbiological activity of soils is also supported by the high level of humus. Thus, the humus is an important indicator of soil fertility. The humus content in the studied soil was 5.4%, typical sod-podzolic heavy loamy soils (3-6%).

Actual acidity is due to the content of free hydrogen ions in the soil solution and is measured on the pH value of the aqueous extract from the soil. In the studied soil reaction is neutral environment (6.1-6.5).

The sum of exchangeable bases (S) – total amount of alkali and alkaline earth cations absorbed by the soil and is able to exchange, depends on soil texture and humus content. In the studied soil, the amount of exchangeable bases – 23, which exceeds typical of sod-podzolic soils a value of 15-20 mEq/100 g of soil.

The cation exchange capacity (PC) is the sum of all the cations absorbed by the soil. It depends on granulometric composition and content of humus. The heavier the soil, the higher PC. In the analyzed soil, the PC is moderately low (15-25 mEq /100 g of soil).

The degree of saturation of soil base (V) shows the percentage of exchangeable bases in the total absorption capacity. It used to evaluate soils in relation to the saturation of bases. In the analyzed soil is high (>90%).

Nitrogen is a part of the composition of organic matter of the soil. Total content of nitrogen in the surface layer of soils varies in the range of 0.10 to 0.85%.

The total content of phosphorus depends on the mechanical composition of the soil and humus. The heavier the texture, the higher the phosphorus content. The content of phosphorus, according to the classification Kirsanov high (151-250 mg/kg) (Kovda, 1988; Kovriga, 2010).

The influence of different forms and doses of fertilizers on the dynamics of growth of the aerial parts of barley. Measure the length of the aerial part was conducted a week after the

start of the experiment. At the first measurement with the lowest value of the length of the aerial part was observed when introducing solids at doses 2.71 and 5.42 g/kg, and 37.0 and 37.8 mm, respectively. The length of the aerial part in the control dimension is slightly above – 40.1 mm. The highest value when making with urea – 55.8 mm, 47.6 and 50.1 mm.

In the second measurement the minimum length of the aerial parts was observed in the control variant and in making pellets of 1.12 g/kg and dry residue 5.42 g/kg. The maximum length of the aerial parts was observed in the urea. The third measurementwas like the first two measurements. The fourth measurementshown the smallest length of the aerial part remained in the version with the introduction of pellets at a dose of 1.12 g/kg – 201.2 mm. The maximum length of options when introducing solids at a dose of 1.36 g/kg (254.1 mm) and control with urea in doses of 0.16 g/kg 0.32 g/kg, 0.64 g/kg – 269.3 mm, 291.2 and 267.5 mm, respectively.

The effect of different doses and forms of fertilizers on biometrics barley. Biometrics – the main parameters characterizing the growth and development of culture. These include the height of the aerial part, root length, weight of aerial parts and roots, etc. According to the data obtained the most effective fertilizer to stimulate growth and increase the weight of the aerial parts of barley are pellets in the dose of 2.20 g/kg, 4.28 g/kg and the dry residue in a dose of 1.36 g/kg. This effect may be caused by a more uniform distribution of solids in the soil, and consequently, the reduction necessary for plant nutrition dose.

The introduction of ureawasalso effective, which compares the use of waste biogas plant. These options have the greatest effect phytostimulation compared to the rest. The least effective dose for the posting turned out to be the pellets at a dose of 1.12 g/kg. This is because contained in the pellets sawdust of coniferous trees has a high ratio of carbon to nitrogen, adverse to food microorganisms, which does not compensate for the nitrogen contained in the pellet in a minimal dose.

The effect of different doses and forms of fertilizers on the effect of phytostimulation. The data indicate that the greatest effect on root length and their weight had pellets at a dose of 2.20 g/kg and the dry residue in a dose 5.42 g/kg. Large mass, but the smaller root length showed the options with the introduction of pellets per dose to 4.28 g/kg of dry residue in a dose of 1.36 g/kg, and the control variant and urea applied at a dose of 0.32 g/kg. The greatest effect of phytostimulation was observed in the variant with urea at a dose of 0.32 g/kg.

The influence of different forms and doses of fertilizers on the cellulolytic activity of the soil. Enzymatic activity of soil can be used as a diagnostic indicator of fertility of different soils, because the activity of enzymes reflects not only the biological properties of soil and their changes under the influence of agroecological factors. The cellulolytic capacity of the soil is one of the indicators of the total activity of soil microorganisms and soil fertility. It can serve as a characteristic of the transformation of organic matter, the involvement of hard to reach forms of carbon in the biological cycle and ultimately determines the level of soil fertility (Secondyou, 2010). According to the data obtained highest cellulolytic activity was observed when you make pellets at a dose of 1.12 g/kg and of dry residue in the dose 5.42 g/kg. Stimulation of activity destroys the cellulose microorganisms can be caused by the presence in these fertilizers movable of copper, zinc, manganese and iron. Lowest cellulolytic activity when urea 0.64 g/kg. Depressing effect of high doses of urea may be caused by excess nitrogen and lack of other nutrients for microorganisms.

Conclusion

On the basis of laboratory experiments with dry residue and pellets of cattle manure on sod-podzolic soil in the laboratory of ecology Department of the Perm state agricultural Academy (Russia), we can draw the following conclusions:

Investigated sod-podzolic soil, heavy granulometric composition, has much humus, a neutral reaction medium, moderately low cation exchange capacity, high degree of saturation with bases characterized by a high content of phosphorus.

The dynamics growth investigation of the aerial parts of barley showed that throughout the period of observation of maximum length reached the plants in the variants with the introduction of solids at a dose of 1.36 g/kg and urea, the lowest indices in the pellets at a dose of 1.12 g/kg.

Investigation of the effect of introducing different forms and doses of fertilizers on biometrics of barley showed the greatest effect on the length and weight of the aerial part from the making of the pellets at a dose of 2.20 g/kg and 4.28 g/kg, dry residue at a dose of 1.36 g/kg. Is also effective was the introduction of urea.The smallest effect on the height and weight of the aerial part had pellets at a dose of 1.12 g/kg. The length and weight of roots of barley greatest effect was influenced by the introduction of dry residue at a dose 5.42 g/kg and pellets at a dose of 2.20 g/kg. The smallest effect was from urea at a dose of 0.64 g/kg.

Investigation shown the greatest phytopromotionaleffect was when you make pellets at a dose 4.28 g/kg of dry residue in a dose of 1.36 g/kg. But, compared with urea, called this effect was less.

The most intensive decomposition of cellulose in the studied soil was observed when depositing pellets of 1.12 g/kg and dry residue 5.42 g/kg. Lowest cellulolytic activity when urea applied at a dose of 0.64 g/kg.

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EFFICIENCY OF MINERAL FERTILIZERS AND BIOLOGICAL NITROGEN ON BARLEY

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Abstract

Use of mineral fertilizers is one of the most important factors determining the size and stability of productivity of crops in Perm Krai. We conducted researches on studying of efficiency of mineral fertilizers and biological nitrogen on productivity and quality of barley at cultivation on a sod-podzolic heavy loam soil. Researches showed that the level of productivity of barley depended on mineral fertilizers and preceding crop. When using a clover of 2 years of use as the it is got the reliable increase of productivity in comparison with barley and a lupine. On a lupine, is traced only the tendency of increase in productivity of barley since the increase in comparison with barley made 0.45 t/ha ($LSD_{0.95}$ = 0.47 t/ha). Mathematical processing of results showed that productivity barley after on barley and on a lupine more depends on nitrogen. On the basis of the results received in experience are developed for each predecessor the production functions characterizing quantitative dependence between productivity of barley and doses of fertilizers. Quality of grain also depended on the studied factors. Best grain of barley is received after clover. Each kilogram of the application fertilizer provided depending on option from 0.3 to 29.7 kg of grain. The maximum payback (29.7 kg/kg) is noted in option with use one nitrogen after barley, additional introduction of phosphorus-potassium fertilizers reduces this indicator. The similar tendency is tracked and on the lupine predecessor. After clover payback of 1 kg of mineral fertilizers is much lower that is explained by a low increase of productivity barley of relatively control.

Keywords: *Mineral fertilizers, biological nitrogen, barley, sod-podzolic soil, yield*

Introduction

The current economic conditions in agriculture do not allow to apply the amount of mineral and organic fertilizers required for crops. Plowing the soil and replacement of perennial vegetation leads to a decrease in revenues of plant residues to the soil, humus mineralization and as a result the degradation of the soil (Mudrykh et al., 2014b). Consequently, one of the urgent problems of agriculture in our region is to find ways to increase the yield of grain by means of alternative sources of organic matter and other nutrients.

According to the Ministry of Agriculture and Food of the Perm region, sown area of barley is about 69 thousand hectares of the total area occupied by the spring grain crops. The average yield of barley in the Perm region in 2014 was 18-22 t/ha. This is a very low yield, as the potential yield of different cultivars of barley is 60-80 t/ha (The Ministry of Agriculture). Achieving this level is only possible if all the elements of barley cultivation technology are followed, while the most important element is to regulate the conditions of mineral nutrition. However, the use of fertilizers in the Perm region remains low. In 2013-2014 14-15 kg of fertilizers has been introduced per hectare of grain field, of which about 5.6 kg was nitrogen. This dose of nitrogen provides only the growth and development of plants in the initial phase. Based on the above statements, the relevant problem of agriculture in our region is to find ways to provide crops with nutrients throughout all the growing season.

The purpose of the research is to evaluate the effectiveness of mineral fertilizers and biological nitrogen on productivity and quality of barley under cultivation on sod-podzolic heavy loamy soil.

Materials and methods

The research was conducted in 2013. Field experiment is laid in a long-term stationary experiment on the field of federal state budgetary scientific institution Perm Agricultural Research Institute in accordance with standard procedure (Dospekhov, 1985) as follows:

Factor A – preceding crop:

A_0 – permanent barley;

A_1 – clover of the 2nd year;

A_2 – blue lupin.

Factor B – mineral fertilizers:

B_0– control (no fertilizers);

B_1 – N_{60};

B_2 – $P_{30}K_{60}$;

B_3 – $N_{60}P_{30}K_{60}$.

There was the triple replication of the experiment variants. Systematic plot allocation in 4 layers. Overall area of experimental plot for Factor A – 300 m^2 (25×12 m), registration plot area – 184 m^2 (23×8 m), for Factor B – 75 m^2 (25×3 m) and 46 m^2 (23×2 m) respectively.

The following fertilizers were used: ammonium nitrate, normal superphosphate, potassium chloride. All fertilizers were applied manually during presowing cultivation. Measurement of barley yield was carried out by a direct method in the phase of full ripeness of grain.

The mathematical processing of the research results was carried out using the Microsoft Excel, STATISTICA 7.0 and MINITAB 14 programs.

The researches were conducted on sod-podzolic heavy loamy soil. The content of humus in the soil varies from very low to low (1.89-2.67%), the pH index from low to medium acid (pH_{KCl} = 4.7-5.42). The content of labile phosphorus is very high (378.7-670.0 mg/kg of soil), exchange potassium from elevated to very high (161.2-428.2 mg/kg of soil).

Weather conditions play an important role in the intake of nutrients from the soil, which subsequently affects the productivity of the crop. Let us consider weather conditions during growing seasons of spring barley compared with the average long-term annual data (Fig. 1).

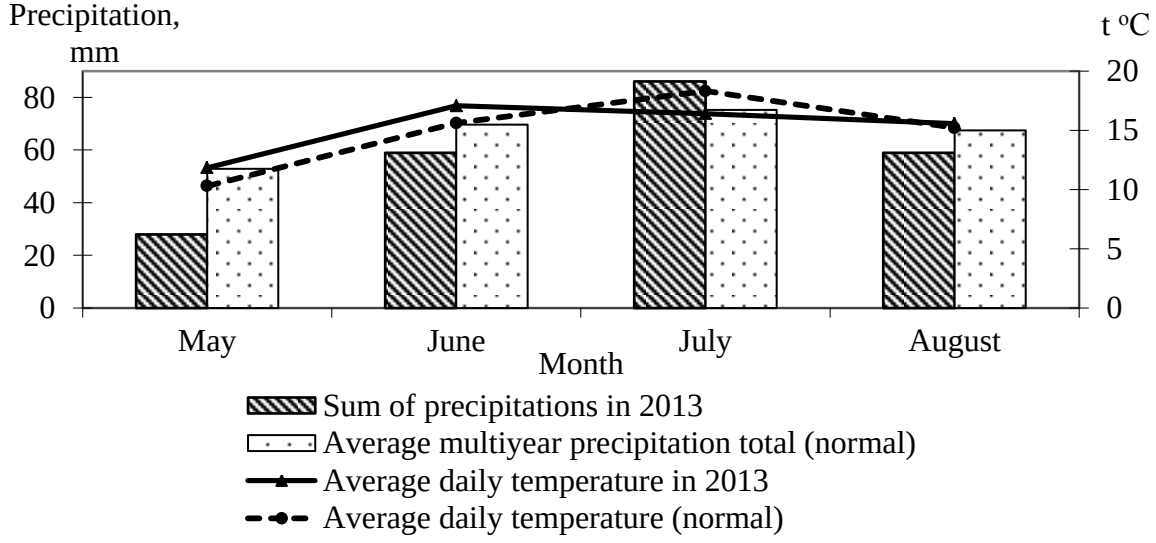


Figure 1. The dynamics of air temperature and precipitation in 2013 and average long-term annual data (according to Perm HMS)

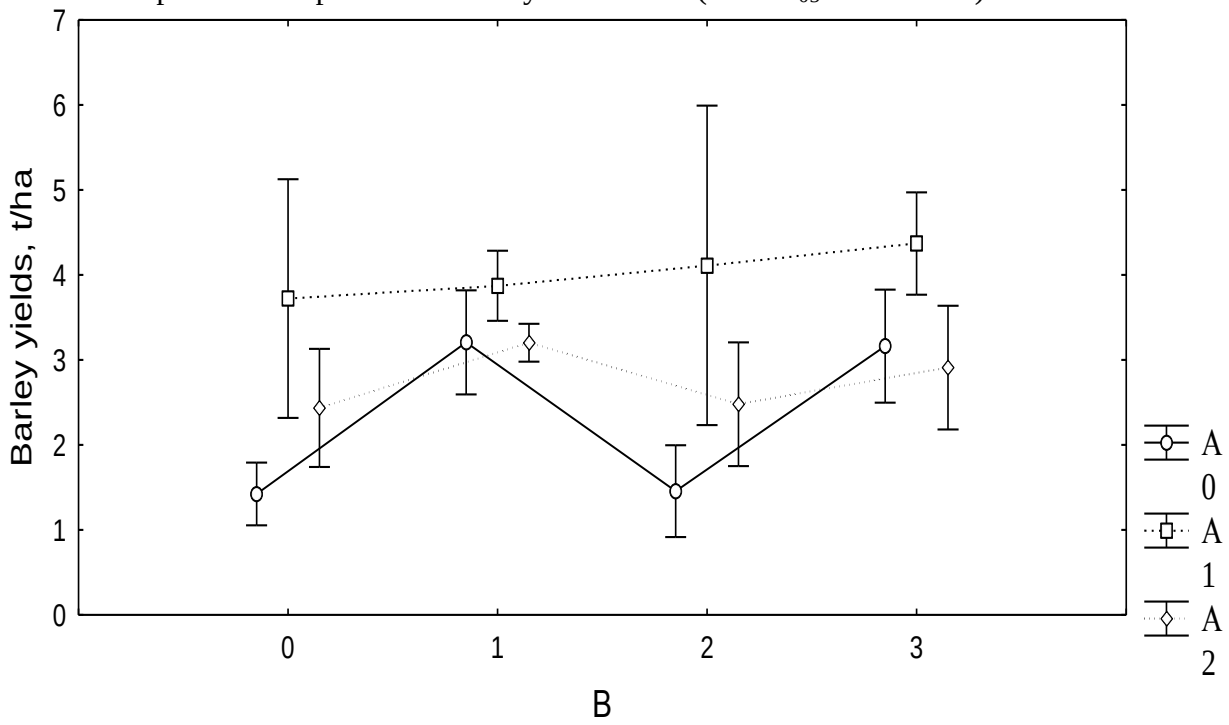
In the first decades of May 2013 there were low average daily air temperature and the abundance of rainfall, which led to the later emergence of seedlings and prolongation of

period from seedlings to tillering. In June, weather conditions were mainly quite favorable for plant growth and development. The tillering phase and establishment of generative organs proceeded in extreme conditions, namely high temperatures and lack of moisture, which further affected the yield of barley. July was characterized by erratic, mostly dry weather, which could lead to lack of grain at certain heads. There were often precipitation including the torrential rains. In this regard, the formation of the barley in July continued rapidly yet growth processes were slow. Weather conditions in August were favorable for harvesting barley. In general, the weather conditions of the vegetation period in 2013 can be described as satisfactory.

Results and discussion

Measurement of barley yield showed that it depends on the fertilizers and preceding crops (Fig. 2).

Considering the main effects of Factor A, we can say that using clover of 2nd year as a preceding crop we obtained significant yield increase compared to permanent barley and lupin. For lupin, in turn, only a tendency to increase the yield of barley can be traced, because increase compared to the permanent barley is 0.45 t/ha (at LSD_{05} of 0.47 t/ha).



Factor A: 0 – permanent barley, 1 – clover of the 2nd year, 3 – blue lupin. Factor B: 0 – control (no fertilizers), 1 – N_{60}, 2 – $P_{30}K_{60}$, 3 – $N_{60}P_{30}K_{60}$ (LSD_{05} for main effects of Factor A – 0.47 t/ha, Factor B and AB – 0.32 t/ha; particular I differences – 0.94, II differences– 0.55 t/ha)

Figure 2. Barley yields, t/ha

Considering the particular differences it should be noted that in the variants without nitrogen fertilizer bean preceding crops have provided a significant increase in yield of barley grain. For example, in the control group of permanent barley we obtained yield of 1.43 t/ha, in clover of the 2nd year – 3.72, in lupin – 2.44 t/ha. When applying nitrogen fertilizer, only clover of the 2nd year has shown significant increase in the version with complete mineral fertilizer.

Evaluation of the main effects of Factor B shows that phosphorus-potassium fertilizers did not provide a significant increase in yield of barley grain. Significant increment (0.8-0.9 t/ha) was

only observed with nitrogen fertilizer in comparison with both the control and with $P_{30}K_{60}$ variant.
Particular second-order differences indicate that the most pronounced increment due to nitrogen fertilizers was obtained by using them with permanent barley – 1.78 t/ha compared to the control and 1.70 t/ha with phosphorus-potassium fertilizers.
The mathematical processing of the results showed that both the permanent barley and lupine yields depend more on nitrogen. The correlation coefficient was 1.000 and 0.942 respectively. Yields of barley with these preceding crops were practically independent of the use of phosphorus-potassium fertilizers. By clover seam, yield was largely dependent on the phosphorus-potassium nutrition (r = 0.903) and to a lesser extent on nitrogen (r = 0.416).
Based on the results obtained in the experiment, production functions describing the quantitative relationship between the barley yield (t/ha) and doses of fertilizers were designed for each preceding crop. These models are as follows (Table 1).

Table 1. Regression models predicting yields of barley

Preceding crop	r	Regression models	R^2
permanent barley	0.96	$У^* = 1.44+0.0291X_1+0.00017X_2$	0.95
clover of the 2nd year	0.98	$У = 3.70+0.00342X_1+0.0148X_2$	0.96
blue lupin	0.65	$У = 2.52+0.100X_1-0.0042X_2$	0.57

*У – barley yield, t/ha; X_1 – dose nitrogen, kg/ha; X_2 – dose phosphorus, kg/ha.

Using these models, we can predict the level of yields of spring barley depending on doses of mineral fertilizers and preceding crops (Table 2).

Table 2. Trend of barley yields and deviation from it

Doses, kg/ha			Barley yields (fact.), t/ha	Trend of barley yields, t/ha	Deviation from trend, %
N	P	K			
Preceding crop – permanent barley (A_0)					
0	0	0	1.43	1.44	1.2
60	0	0	3.21	3.19	-0.6
0	30	60	1.46	1.45	-0.8
60	30	60	3.16	3.19	0.9
Preceding crop – clover of the 2nd year (A_1)					
0	0	0	3.72	3,70	-0,6
60	0	0	3.87	3,91	0,8
0	30	60	4.11	4,14	0,7
60	30	60	4.37	4,35	-0,5
Preceding crop – blue lupin (A_2)					
0	0	0	2.44	2,52	3,3
60	0	0	3.21	3,12	-2,7
0	30	60	2.48	2,39	-3,6
60	30	60	2.91	2,99	2,8

According to the obtained data, the deviation of calculated yield levels from the actual ones is low and ranges from -3.6 to 3.3 %. This indicates the high accuracy of the model, i.e. this model can be used to predict the level of barley yield depending on the preceding crops and used doses of mineral fertilizers.
The efficiency of fertilizer use is assessed by the agronomic and economic payback. To improve the economic efficiency of production it is necessary to reduce the cost of

production, increase quality and sales of products at a higher price. To reduce production costs it is necessary to apply modern production technology. Therefore, according to the problem of this study we shall define the efficiency of applying fertilizer on barley in various preceding crops in economic terms.

Significant yield increase due to mineral fertilizers was achieved in variants with doses N_{60} and $N_{60}P_{30}K_{60}$ at permanent barley, $N_{60}P_{30}K_{60}$ after clover of the 2nd year and N_{60} after lupin. Based on calculations, we can conclude that in the variant with the use of nitrogen fertilizers in dose N_{60} for the permanent barley the highest levels of net income (5826.72 rub.) and profitability (120 %) were achieved. In the variant with complete mineral fertilizer the profitability is lower due to increased costs for fertilizer use. The use of nitrogen fertilizer on barley after clover of the 2nd year was not profitable, as the cost of the use of fertilizers exceeds the value of increment. Introduction of nitrogen fertilizer on barley after blue lupine also led to obtaining a positive economic effect, but its profitability will be lower compared to the N_{60} version for the permanent barley. So, at the same cost of use of nitrogen fertilizer, the largest conditional net income from the use of nitrogen fertilizers was gained on the permanent barley – 5826.72 rubles. That is many times more than in the cultivation of barley after lupine.

The annual change in prices for the products and expense items does not allow comparison of payback for measures applied, so the efficiency of fertilizer use is determined by agronomic evaluation (Mudrykh et al., 2014a).

We calculated the agronomic payback, the results are shown in Table 3. The used fertilizers provided different barley grain yield increase, which affected the payback of 1 kg of active material.

Table 3. The agronomic efficiency of mineral fertilizer application

Variants	The yield, t/ha	Return 1 kg of NPK increase in yield, kg/kg	The costs NPK on increase yields grain, kg/t
Preceding crop – permanent barley (A_0)			
0	1.43	–	–
N_{60}	3.21	29.7	33.7
$P_{30}K_{60}$	1.46	0.3	3000.0
$N_{60}P_{30}K_{60}$	3.16	11.5	86.7
Preceding crop – clover of the 2nd year (A_1)			
0	3.72	-	-
N_{60}	3.87	2.5	400.0
$P_{30}K_{60}$	4.11	4.3	230.8
$N_{60}P_{30}K_{60}$	4.37	4.3	230.8
Preceding crop – blue lupin (A_2)			
0	2.44	-	-
N_{60}	3.21	12.8	77.9
$P_{30}K_{60}$	2.48	0.4	2250.0
$N_{60}P_{30}K_{60}$	2.91	3.1	319.1

Each kilogram of applied fertilizer, depending on the variant, provided from 0.3 to 29.7 kg of grain. Maximum payback (29.7 mg/kg) was observed in the variant with nitrogen only in permanent barley, the additional introduction of phosphorus-potassium fertilizer reduces the figure. A similar trend can be seen for lupine as a preceding crop. By clover seam, payback of 1 kg of mineral fertilizers is significantly lower due to the low yield increase relative to the control variant.

Due to the fact that when determining the fertilizer needs for the crop the fundamental parameter is the amount of nutrients required for the yield unit formation, we found it

necessary to determine this value (Table 3). The amount of fertilizers required for the 1 t increase of productivity also varied greatly depending on the variant, the range of variation was 33.7-3000 kg. The amount of fertilizer required to gain an increment of 1 t of barley yields has the same trend, but in the reverse order. At a higher payback of mineral fertilizer its consumption at the formation of the yield unit growth decreased.

Conclusion

Based on the conducted researches to study the effectiveness of mineral fertilizers and biological nitrogen on yield and quality of barley under cultivation on sod-podzolic heavy loamy soil of the experimental field of the state scientific institution "Perm Agricultural Research Institute", the following conclusions can be made:

1. For barley the best preceding crops is a red clover of the 2nd year and lupin.
2. Production functions allow to predict the level of productivity depending on the preceding crops and doses fertilizer.

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TESTING CONSTITUTION AND REPRODUCTIVE POTENTIAL OF SVRLJIG ZACKEL SHEEP FOR ORGANIC PRODUCTION IN EAST SERBIA

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Abstract

Eastern Serbia region, especially Homolje territory is an important natural resource of a great potential for organic sheep production. Its long tradition of sheep breeding and the quality of lamb meat and dairy products has become well-known. Svrljig type of Zackel sheep is traditionally reared in the hilly - mountain region of Eastern Serbia. It is well adapted to environmental conditions and sustainable breeding systems. Bearing in mind that autochthonous breeds are preferable for organic production due to the fact that they are well adapted to habitat and due to their genetic tolerance to diseases, the autochthonous Svrljig sheep population reared in Homolje territory was chosen for this research. Examination of health status, body score condition, reproductive traits in a population of 342 ewes and growth traits of 412 lambs reared in sustainable livestock farming was performed. Reproductive and lamb growth traits, litter size at weaning (LSW), total litter weight at birth (TLWB), total litter weight at one-month (1TLMW) and total litter weight at weaning (TLWW) were analyzed. Results of health status, basic ewe reproductive traits and lamb growth performance show that autochthonous Svrljig type of Zackel sheep has robust constitution and potential to be the breed of choice for organic production in the hilly - mountain region of Eastern Serbia.
Key words: *Zackel sheep, health status, production traits*

Introduction

One of the primary elements in the organization of organic production is the choice of suitable breeds. Each breed and strain of domestic animals were created through a process of interaction between genome and environment, along with adjustment imposed by man. According to the legal regulations of the methods of organic livestock production (2010), animal species and breeds that are adapted to local growing conditions and resistant to disease are envisaged to be used in organic production (Cojkić et al., 2014.; Savić et al., 2014). When choosing the animals, advantage is given to domestic (autochthonous) animal breeds and strains (Petrović et al., 2009.).

Autochthonous breeds, whose microevolution occurred under the influence of ambient selection factors represent a unique source of genetic variability that enable adaptation to the new conditions and may enable the survival of the population under adverse action of pathogenic microorganisms Organic production legal regulations set high standards which specify the conditions for breeding animals in self-sustaining systems. Animal breeding is based on physiological and ethological needs of the animals. But from the economic aspect of production, it is important to evaluate the reproductive and production potential of the breed.

Reproduction of animals in organic production is carried out through natural mating or artificial insemination. Other methods of biotechnology in reproduction such as embryo transfer, MOET and hormonal induction of estrus are not allowed.

Organic farming is a specific, innovative aspect of production, which brings significant innovations, especially the one relating to veterinary supervision. The regulation is quite strict when it comes to the prohibition of preventive treatment. Health care in organic livestock production is based on the principle of disease prevention. Legislation in this area emphasizes breeding of genetically resistant animals with the improvement of environmental conditions and care.

Economical use of well-adapted indigenous animals is achieved by preserving the diversity of domesticated animal species for the selection procedure and as a source of the gene pool that can confront unpredictable immune challenges.

A good adaptation of the animals to the local environmental conditions allows a maximal expression of their production potential. This is reflected in the quantity and quality of milk, meat, as well as reproductive features for a given species, breed or strain of animals. In sheep breeding the production is mainly focused on the production of meat. Consequently, the goal is to get a greater number of live, vital lambs per ewe, and for lambs to have some better feed conversion and therefore better growth.

This paper presents robustness-related traits of Svrljig type of Zackel sheep which are breeding in several municipalities of Eastern Serbia. The aim of this study was to investigate the potential of the locally adapted Zackel sheep, and the possibility of rational utilization and conservation of this strain of sheep as an important element for rural agro-economic development.

Materials and methods

Animals

In total, 342 ewes, 3 to 5 years old, and 412 lambs of Svrljig type of Zackel sheep breed, traditionally reared in Eastern Serbia, Homolje region, were examined for health status, body score condition, reproductive and lamb growth traits. The evaluation was performed during the winter and spring. During the spring and summer, the sheep were grazed on mountain natural pastures. On the other hand, during the autumn and winter the diet of ewes was based on hay and concentrate. The lambs remained with their mother until weaning at 90 days and fed ad libitum with hay and concentrate with 18% of protein.

Studied traits

Reproductive and lamb growth traits analyses included litter size at weaning (LSW), the sum of the birth weight of all lambs born per ewe lambed (TLWB- total litter weight at birth), the sum of the weights off all lambs on one month of age (1TLMW - total litter weight at 30 days) and the sum of the weights off all lambs weaned per ewe lambed (TLWW - total litter weight at weaning-90 days).

Statistical analysis

The software package Prism Pad v. 6.0 (Graph Pad Software Inc., San Diego, CA, USA) was used for statistical calculations. Reproductive and lamb growth traits were presented by descriptive statistical parameters. Student t-test was used to examine the differences of weight between male and female lambs, twins and single lamb of every measured period (TLWB, 1TLMW, TLWW).

Results and Discussion

The first stage in this study was the evaluating the body condition scoring of sheep. The results of this analysis have shown that all examined sheep were right BSC considering the stage of production.

Monitoring of sheep health carried out based on the Program of animal health protection measures. Based on that, animals are monitoring for Brucellosis (B. melitensis, B. ovis), transmissive spongiform encephalopathy (TSE) and the diagnostictestsin case ofabortions. Percentage of abortionsin the herdwas not asignificant. No one abortionwas notcaused byinfectiousdisease (brucellosis, leptospirosis, listeriosis, and Q fever). Analysis of the reproductive characteristics of Svrljig sheep shown regular fertility. Reproductive traits were in accordance with breed standards.
The results of the constitutional and reproductive traits of ewes as well as body weight of lambs at birth (TLWB), on 30 days (1TLMW) and 90 days (TLWW) are shown in Table 1.

Table 1. Summary of descriptive statistics for constitutional, reproductive and lambs growth traits.

	Lambs			**Ewes**	
	TLWB (kg)	1TLMW (kg)	TLWW (kg)	Body weight (kg)	LSW (lamb)
$\bar{x} \pm SE$	3.39 ± 0.03	8.90 ± 0.04	26.62±0.11	56.25 ± 0.17	1.20 ± 0.02
SD	0.58	0.78	2.16	3.42	0.40
IV	2.0 – 4.7	6.9– 13.0	22.0 – 35.0	48.0 – 67.0	1.0 – 2.0
CV	17.28%	8.78 %	8.10%	6.09%	33.47 %

TLWB- total litter weight at birth, 1TLMW- total litter weight at one-month age, TLWW - total litter weight at weaning, LSW - litter size at weaning

The average TLWB, 1TLMW and TLWW were 3.39 kg, 8.90 kg and 26.62 kg, respectively. In the study of Caro Petrović et al. (2012), performed on the Svrljig type of Zackel sheep, the average birth weight of lambs and weight at 30 days was higher than in our study. On the other hand, the average weight of lambs at 90 days was lower. Indirect lamb selection based on TLWW could be efficient for the studied traits (Roshanfekr et al., 2015).
The mean body weight of ewes on lambing, measured was 56.25 kg. The result is in agreement with study which reported that birth weights of lambs depend on the weight of ewes (Petrović et al., 2011).
In this study litter size at weaning, LSW was 1.20, which is an agreement with Zackel breed characteristics. Differences in reproductive performance could be explained by many factors like genetics, maternal behavior of ewes at different ages, nursing and the differences in maternal effects (Roshanfekr et al. 2015).
The influence of sex of lamb, birth type and lambs` body weight are presented in this study.

Table 2.The differences in body weight of lambs, based on sex and birth type

	Male lamb (n=38)	Female lamb (n=374)	Singles (n=274)	Twins (n=138)
TLWB	4.14±0.39^{AA}	3.01±0.55^{AA}	3.76±0.27 ^{BB}	2.64±0.23 ^{BB}
1TLMW	10.14±1.22 ^{AA}	8.78±0.60 ^{AA}	9.05±0.79 ^{BB}	8.60±0.67 ^{BB}
TLWW	30.73±2.72 ^{AA}	26.14±2.00 ^{AA}	26.66±3.01	26.15±1.91

TLWB- total litter weight at birth, 1TLWM- total litter weight at one-month age, TLWW - total litter weight at weaning: AA, BB - ($P < 0.001$)

In the Table 2. The average body weight at birth (TLWB), at one month of age (1TLMW) and at weaning (TLWW) of both sexes of lambs and birth type were presented.
The differences in TLWB, TLW1 and TLWW between male and female lambs were statistically significant ($P < 0.001$). Likewise, the differences in TLWB, 1TLMW between singles and twins were statistically significant ($P < 0.001$). On the other hand, there was difference in TLWW between birth type, but not statistically significant ($P > 0.05$).
This is in agreement with the results of the other researchers (Abdullah and Tabbaa, 2011.). Gamasaee et al. (2010) stated that the effect of birth type was significant on birth weight of lambs and can be explained by limited uterine space and nutrition of lamb during pregnancy. The difference in weight between single and twin lambs increased from birth to weaning, which could be attributed to that singles were more capable of suckling their mothers than twins. The growth traits are important in productivity and are the major selected traits in sheep breeds. They are influenced by genetic and environmental factors as shown in numerous studies (Behzadietal., 2007, Dass et al., 2008.).

Conclusion

Homolje territory is an important natural resource of great potential, with specific characteristics of HNV (High Nature Value) region. Flora and fauna of Homolje region is particularly rich and abundant in many plant and animal species which, some of them being relict. Under agricultural land is 36 880 ha or 48% of the territory. Of that 42.9% of meadows, 26% of pastures and 31.1% is arable land. Meadows and pastures represented from the lowest level parts to the highest parts of the mountain ranges, which is a great potential for organic sheep production.
Considering the results Svrljig sheep is identified as a good potential for further development of organic sheep husbandry in East Serbia hilly mountain ecoregions. Svrljig sheep reared in a sustainable production system, and since it uses well natural pastures and meadows, its diet is based on existing plant resources. For achieving better productive results of Svrljig sheep, an integrated approach is necessary. The approach must contain all aspects of sheep keeping, feeding, breeding and disease prevention, as well as pasture management.

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Review paper
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SEASONAL CHANGES OF MICROBIAL POPULATION IN MAIZE AND SOYBEAN RHIZOSPHERE UNDER CONVENTIONAL AND ORGANIC GROWING SYSTEMS

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Abstract

The aim of this study was to compare abundance of microorganisms in the rhizosphere of maize and soybean grown under conventional and organic management. The trial was set up on the chernozem soil at the experimental field of Bački Petrovac, Institute of Field and Vegetable Crops, Novi Sad. Rhizosphere samples were collected in two sampling terms during 2014 (July 7 and August 14) and analysed by the indirect dilution method followed by plating of soil suspension on selective nutritive mediums: soil agar for the total number of microorganisms, meat peptone agar for the number of ammonifiers, Waksman-Carey medium for the number of cellulolytic microorganisms, synthetic medium for the number of actinomycetes, Czapek Dox agar for the number of fungi, and N-free medium for the number of azotobacters and free N-fixers. The results showed significant differences in microbial abundance between plant species, growing systems and sampling terms. Significantly higher number of microorganisms was found in rhizosphere of soybean compared to maize, as well as in organic growing system compared to conventional. Significant differences between sampling terms were recorded for the number of ammonifiers, azotobacters, fungi and actinomycetes, while the number of most tested microbial groups was higher in the second sampling term.

Keywords: *maize, microbial abundance, organic and conventional growing system, rhizosphere, soybean*

Introduction

Agricultural intensification as one of the greatest threats to global biodiversity, leads to large-scale ecosystem degradation and loss of productivity in the long term (Convention on Biological Diversity, 2010). Management practices (tillage, cropping rotation, use of pesticides and mineral fertilizers, irrigation) strongly influence the quality and fertility of agricultural soils and consequently, the size, composition, and function of the soil microbial community (Shannon et al., 2002). In turn, despite of their small volume in soil, microorganisms play a definitive and very useful role in soil fertility through the cycling of carbon, nitrogen, phosphorus, sulfur, and decomposition of organic residues (Nannipieri et al., 2003; Singh et al., 2011). In addition to the effect on nutrient cycling, microorganisms also affect the physical properties of the soil via production of extracellular polysaccharides and other cellular debris, and thereby help in maintaining soil structure as well as soil health (Bastida et al., 2008).

Conventional farming systems have been associated with loss of soil fertility, soil erosion and ground water pollution (Pimentel et al., 2005). Low-input systems such as organic farming, substantially reduce the use of synthetic fertilizers, pesticides, energy and mechanic stress, and mitigate these negative impacts in order to improve sustainable production (Gomiero et al., 2011). Earlier studies have shown that incorporation of organic amendments increase biomass, activity and diversity of soil microorganisms (Gelsomino et al., 2004; Girvan et al., 2004). According to Hartmann et al. (2015), fertilization scheme, the application and quality

of organic fertilizers in particular, is the major determinant of microbial diversity. The addition of animal or green manures on organic plots provides a significantly greater input of organic carbon which increases microbial population (Reeves, 1997).

Microbial processes within the rhizosphere of crop plants are crucial to agriculture. The relation of soil microbial community to cropping system, yield, and soil quality is unclear at present. Seasonal inputs of crop roots, rhizosphere products, and crop residues significantly altered soil microbial biomass and mineralizable C and N of this soil, illustrating the dependence of N dynamics on short-term C inputs and association of soil C/N ratio with changes in microbial community composition across different treatments (Carney and Matson, 2012). Beside management practices and soil environmental factors, the diversity and composition of microbial community in the rhizophere also depend on several other factors including plant species (Mahmood et al., 2005). Plants can influence composition and stimulate activity of soil microorganisms by producing labile carbon compounds through root exudation (Marschner et al., 2004). Valid evaluation of soil quality requires better understanding of short- and long-term responses of key biochemical and microbiological soil properties to seasonal changes and types of management practice in the cultivation of various crops.

Therefore, the aim of this study was to examine the seasonal changes in rhizosphere microbial population of maize and soybean grown under conventional and organic growing systems.

Materials and Methods

The trial was set up on the chernozem soil at the experimental field of Bački Petrovac, Institute of Field and Vegetable Crops, Novi Sad. Rhizosphere samples were collected in two sampling terms during 2014 (July 7 and August 14) and analysed by the indirect dilution method followed by plating of soil suspension on selective nutritive mediums: soil agar for the total number of microorganisms (dilution 10^7), meat peptone agar for the number of ammonifiers (dilution 10^6), Waksman-Carey medium for the number of cellulolytic microorganisms (dilution 10^5), synthetic medium for the number of actinomycetes (dilution 10^4), Czapek Dox agar for the number of fungi (dilution 10^4), and N-free medium for the number of azotobacters (dilution 10^2) and N-fixers (dilution 10^6) (Jarak and Đurić, 2006). Incubation temperature was 28°C, while incubation time depended on the tested microbial group. All microbiological analyses were performed in three replications, and the average number for all samplings was calculated per 1.0 g of absolutely dry soil. The results were analyzed in accordance with three–way model of analysis of variance (ANOVA) using Statistica software (StatSoft Inc. 2012), followed by mean separation according to Fisher's LSD test (Steel and Torrie, 1980).

Results and Discussion

In a long-term field trial in which organic and conventional growing systems were compared, a significant influence of organic agriculture on microbial biomass, diversity and microbial community structures was reported (Esperschütz et al., 2007). Our research focused on comparison of microbial abundance between rhizosphere of maize and soybean grown under conventional and organic management.

At the first sampling term, the number of examined microbial groups in soybean rhizosphere was higher in the organic farming system. Significant differences between systems were recorded for the total number of microorganisms, number of ammonifiers, free N-fixers and cellulolytic microorganisms. At the second sampling term, higher microbial abundance was also obtained in the organic farming system, except for total and free N-fixers, while significant differences were observed within the number of ammonifiers, azotobacters and cellulolytic microorganisms (Table 1). In study of Das and Dkhar (2012), addition of organic

amendments affected the soil physicochemical properties, which in return affected the microbial characteristics in soybean rhizosphere.
The number of microorganisms in maize rhizosphere, was higher in organic management system at the first term of sampling, while significant differences between the systems were recorded only for the number of ammonifiers.
At the second sampling term, for all tested groups except ammonifiers and free N-fixers exhibited the highest number in the organic management system, while significantly higher number between systems was recorded only for the number of actinomycetes and cellulolytic microorganisms (Table 1). Similar results were reported by Orr et al. (2010), indicating that the larger number of free nitrogen-fixers in conventional system was obtained due to higher concentrations of phosphorus from mineral fertilizers. On the contrary, Buyer and Kaufman (1997) showed that total number and diversity of microorganisms in maize rhizosphere were not significantly different for conventional and low-input systems suggesting that conventional agricultural practices may maintain high indices of microbial diversity in the rhizosphere.

Table 1. Number of microorganisms in rhizosphere of soybean and maize

Number of microorganisms in 1 g of absolutely dry soil (CFU g^{-1})					
Microbial group	Growing system	Organic		Conventional	
	Sampling/Plant	I	II	I	II
Total microbial number x10^7	Soybean	155.53^{a}	146.22^{a}	76.19^{bc}	159.63^{a}
	Maize	105.67^{abc}	117.92^{ab}	49.62^{c}	65.01^{bc}
Ammonifiers x 10^6	Soybean	99.47^{b}	162.13^{a}	40.85^{cd}	56.66^{c}
	Maize	60.44^{c}	44.82^{cd}	18.49^{d}	51.34^{cd}
Azotobacters x 10^2	Soybean	122.10^{bc}	198.34^{a}	90.83^{cd}	145.49^{b}
	Maize	25.79^{e}	47.18^{de}	7.81^{e}	12.24^{e}
N-fixers x 10^6	Soybean	106.46^{a}	95.43^{ab}	58.78^{bc}	99.19^{ab}
	Maize	72.81^{abc}	73.14^{abc}	34.52^{c}	73.41^{abc}
Fungi x 10^4	Soybean	11.62^{a}	11.88^{a}	6.01^{ab}	8.65^{ab}
	Maize	3.41^{b}	10.63^{a}	3.14^{b}	6.99^{ab}
Actinomycetes x 10^4	Soybean	19.48^{abc}	30.87^{ab}	2.24^{c}	17.28^{abc}
	Maize	6.85^{bc}	31.90^{a}	1.58^{c}	2.83^{c}
Cellulolytic microorganisms x 10^5	Soybean	30.39^{a}	31.22^{a}	8.16^{c}	14.95^{bc}
	Maize	5.73^{c}	24.46^{ab}	5.55^{c}	6.62^{c}

Means with the same letter are not significantly different at the P = 0.05 level of significance

This research showed significant differences in microbial abundance between plant species, growing systems and sampling terms (Table 2). Higher microbial number was recorded in soybean rhizosphere compared to rhizosphere of maize. Significant differences in microbial abundance were found between plant species, for all microbial groups except actinomycetes. These results are in agreement with those of Carney and Matson (2012) who revealed that microbial community composition differed between plant species. Similarly, significant differences in the soil microbial biomass were not visible during the wheat growing season, as a result of application of mineral and organic fertilizers, whereas fertilizer application significantly increased this parameter during the maize growing season (Mahmood et al., 2005).
Growing system demonstrated a significant impact on the total number of microorganisms, number of ammonifiers, free N-fixers, actinomycetes and cellulolytic microorganisms.

Significantly higher number of microorganisms in the organic farming system compared with conventional was also determined by Mrkovački et al. (2012).
However, their study reports the higher number of microorganisms in rhizosphere of maize compared to soybean, which is contrary to our results, and indicates that variations in the number of microorganisms depend on the year of research. Studies of Kong et al. (2011) confirmed the highest total microbial biomass in the organic management, and similar values between the conventional and low-input systems, while Bettiol et al. (2002) obtained similar effects of organic and conventional growing system on soil microbial populations. Various findings confirmed that stimulative effect of organic amendments on biomass, activity and diversity of soil microorganisms depends on numerous factors, such as soil type, plant species, soil management practices and other environmental variables. Franzluebbers et al. (1994) indicated that crop management strategies can affect the short-term dynamics of the active C and N pools of soil organic matter by altering the timing, placement, quantity, and quality of crop root and residue input, as well as nutrient status and environmental conditions. Fließbach and Mäder (2000) also interpreted higher microbial biomass in soils of the organic systems as an enhanced decomposition of the easily-available light fraction pool of soil organic matter with increasing amounts of microbial biomass.

Table 2. Effect of plant species, growing system and sampling term on microbial population

Number of microorganisms in 1 g of absolutely dry soil (CFU g^{-1})						
Microbial group	Plant species		Growing System		Sampling term	
	Soybean	Maize	ORG	CON	I	II
Total microbial number $x10^7$	134.39	84.59	131.33	87.61	96.75	122.20
	**		**		NSD	
Ammonifiers x 10^6	89.78	43.77	91.72	41.84	54.79	78.73
	***		***		**	
Azotobacters x 10^2	139.19	23.25	98.35	64.09	61.63	100.81
	***		**		**	
N-fixers x 10^6	89.97	63.47	86.96	66.47	68.14	76.13
	*		NSD		NSD	
Fungi x 10^4	9.54	6.04	9.38	6.20	6.04	9.54
	*		NSD		*	
Actinomycetes x 10^4	17.47	10.79	22.27	5.98	7.54	20.72
	NSD		*		*	
Cel. microorganisms x 10^5	21.18	10.59	22.95	8.82	12.46	19.31
	**		***		NSD	

NSD indicates no significant difference at the P = 0.05 level of significance; *, ** and *** indicates significant differences at the P < 0.05, P < 0.01 and P < 0.001 levels

Significant variations in microbial number in relation to the growing season were recorded for the number of ammonifiers, azotobacters, fungi and actinomycetes, while higher abundance was obtained at second sampling term. Shi et al. (2013) recorded seasonal fluctuations in soil microbial biomass C and N, in dehydrogenase and alkaline phosphomonoesterase activities, and in total phospholipids fatty acid (PLFA) level under different tillage and phosphorus management practices, concluding that soil environmental factors and tillage had a greater effect than fertilization on microorganisms (biomass and activity) and community structure. Bossio et al. (1998) observed sustained increases in microbial biomass resulting from high organic matter inputs in the organic and low-input systems that were significantly different during growing season. Same authors ranked the relative importance of various environmental variables in governing the composition of microbial communities in the following order: soil

type > time > specific farming operation (e.g. cover crop incorporation or sidedressing with mineral fertilizer) > management system > spatial variation in the field.

Conclusion

The increase in the number of microorganisms in rhizosphere of two different crops grown under organic management system confirm the positive effect of this agricultural practice on soil microbial population and biological health of soil compared to conventional management.

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EFFECTS OF ORGANIC FARMING ON SOIL COMPACTION

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Abstract

Soil compaction, an important dynamic and physical property of soil, is represented by the force needed for the compression of soil by the working components of an instrument, and can be quantitatively expressed as MPa. The use of heavy machinery and a large number of cultivation practices increase soil compaction. When soils are subjected to external pressure, soil compaction (increase in soil density) occurs, during which the liquid and gas phases of soil are partly or completely extracted. Anaerobic conditions and water stagnation in the active part of rhizosphere can also occur, resulting in the constrained development of the root system, unwanted chemical processes, anaerobic and slow organic matter decomposition. Soil compaction was tested on agricultural plots under certified organic and conventional farming systems, as part of the project supported by the Ministry of Agriculture of the Republic of Serbia. Regardless of their utilization, a high level of soil compaction was generally observed in soils examined at the territory of Central Serbia, caused by high content of clay fraction in the mechanical composition of soil. Identical values of soil compaction were found in the initial 10 cm layer (at the optimal level of 1,0 - 2,5 MPa) both in soils under organic and conventional farming, which can be explained by timely soil management. With the increase in soil depth, compaction of soils under organic farming was lower compared to soils under conventional farming. This result is attributed to higher content of organic matter which was determined in this research.

Keywords: *compaction, organic and conventional farming, soil*

Introduction

Organic farming is a system of ecological management which promotes and improves biodiversity, circulation of matter, biological activity and soil fertility. One of the prime principles of organic management is preservation and increase in soil fertility level (Republic of Serbia, 2010). Organic farming is one version of a sustainable management system, which uses crop rotation, intercropping, mulches, green manure, organic waste, and above all, integration of plant production and animal husbandry.

Considering the principles of organic production, this system of production is expected to ensure preservation and improvement of soil properties, and prevent soil degradation (Nešić et al., 2014).

Soil compaction is one of the main problems of modern agriculture. Increased soil compaction is caused by overuse of heavy machinery for tilling, harvesting, and transport on excessively wet soils, intensive management practices, improper crop rotation and unsuitable soil use. Compaction is defined by increased soil firmness and decreased fertility, caused by the deficiency in soil supplies and inaccessibility of water and plant nutrients. Tilling compacted soils results in increased energy costs, with decrease in yield and quality of crops grown on compacted soils. Furthermore, tilling compacted soils results in runoff and destruction of agricultural machinery, ultimately resulting in increased costs of production on these soils. Compacted soils are also unsuitable as habitats for soil flora and fauna. Since the soil compaction process and its effects on physical characteristics, plant growth, chemical and

ecological processes are still inadequately researched, this study focuses on causes and effects of compaction occurring on agricultural soils, and the possible solutions to this problem (Gajić, 2006).

The aim of the study is to review the effects of organic and conventional farming systems on soil compaction.

Materials and methods

The research was conducted on five locations in Central Serbia. Locations were chosen based on the concentration of plots under organic farming (data taken from the database of Serbia Organica). Area reconnaissance, field survey, and soil sampling were performed in the period from August to October 2013.

The tested soil plots under organic and conventional farming systems are nearby, and a after a review of field reports and through conducting a producers' survey, it was confirmed that the same tilling operations had been applied on the plots during a longer period of time.

Soil compaction was determined with the use of “Penetrologger – Eijkelkamp Agrisearch Equipment” supported by software “Eijkelkamp PenetroViwer Software” . The machine operates on the principle of measuring soil resistance to penetration of the working cone (expressed as MPa (1 MPa = 100 N/cm^2)). Penetration is performed through the opening on the reference metal board on the surface, whose function is to reflect the signal of the ultrasound sensor, precisely showing the depth of measurement. Reference board also serves to emit the signal which controls the speed of penetration. Soil compaction is determined up to 80 cm of soil depth. Total of 100 samples were collected at 14 localities, from the plots under both certified organic and conventional farming systems (Figure 1).

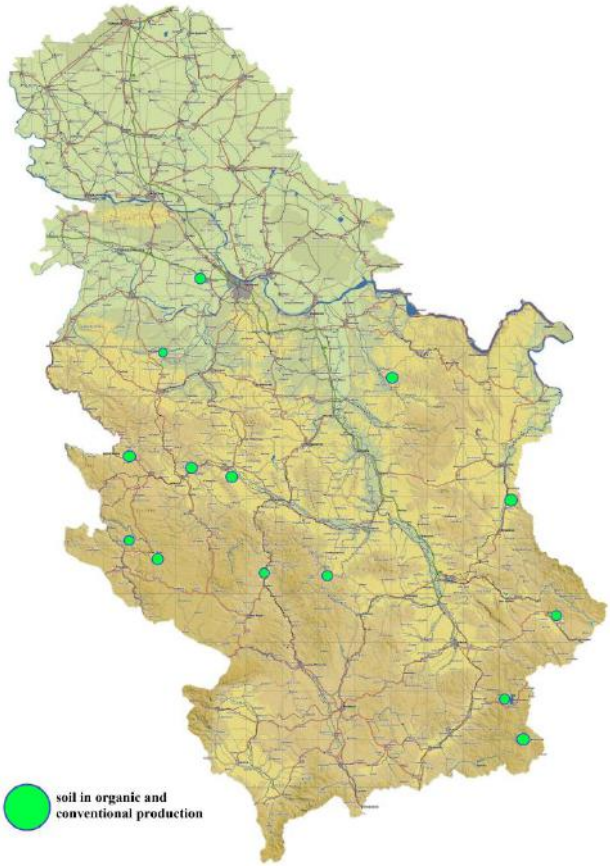
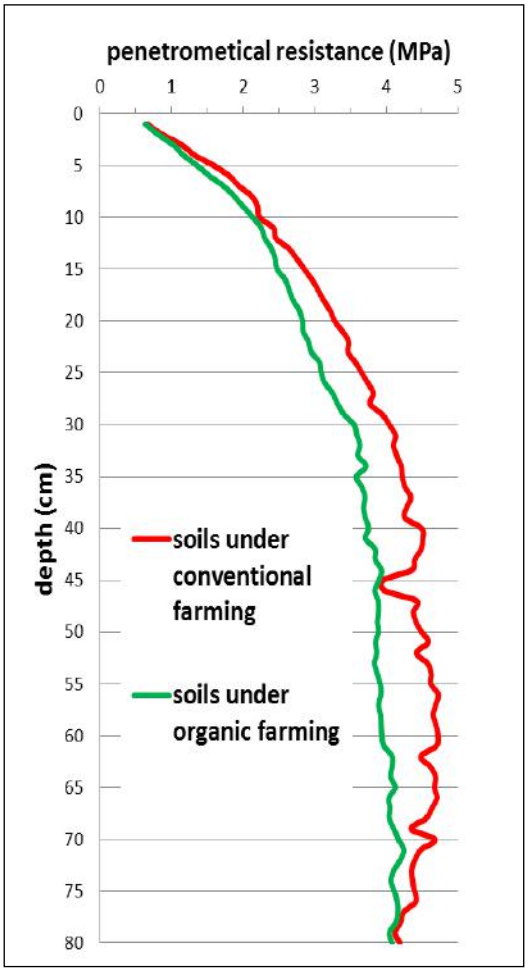


Figure 1 – Locations of plots under organic and conventional farming

For the purpose of conducting analyses within this study, soils were sampled in disturbed condition using the agro-chemical probe at two soil depths (0-30 and 30-60 cm).
The collected samples were tested for soil mechanical composition (pipette method, sample preparation using Na-pyrophosphate according to Thun) and humus content (Turin method), at the Laboratory for Soil and Agroecology at the Institute of Field and Vegetable Crops. The research applied standard methods of the Laboratory accredited by the Serbian Accrediation Body Акредитационог according to the standard SRPS ISO/IEC 17025:2006.

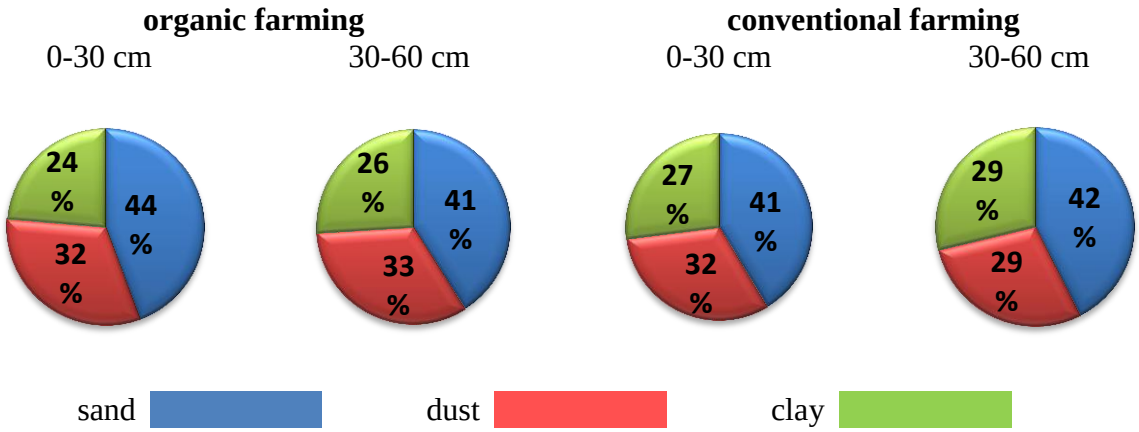
Results and discussion

Observed values of soil compaction (Graph 1) at the depth of 10 cm were classified as moderately or highly compacted soil (according to reference values used to estimate the relative compaction of a soil (Birkás, 2008).



Graph 1 – Compaction (penetrometric resistance) of soils

The obtained high values result from heavier mechanical composition of the tested soil (Graph 2), which is in accordance with the results of Baham (2005), Gajić et al. (2005), and Hettiaratchi (1987). These authors state that mechanical composition of soil is in direct proportion to the intensity of soil compaction, hence heavy soils (soils with a higher clay content) are more compacted.

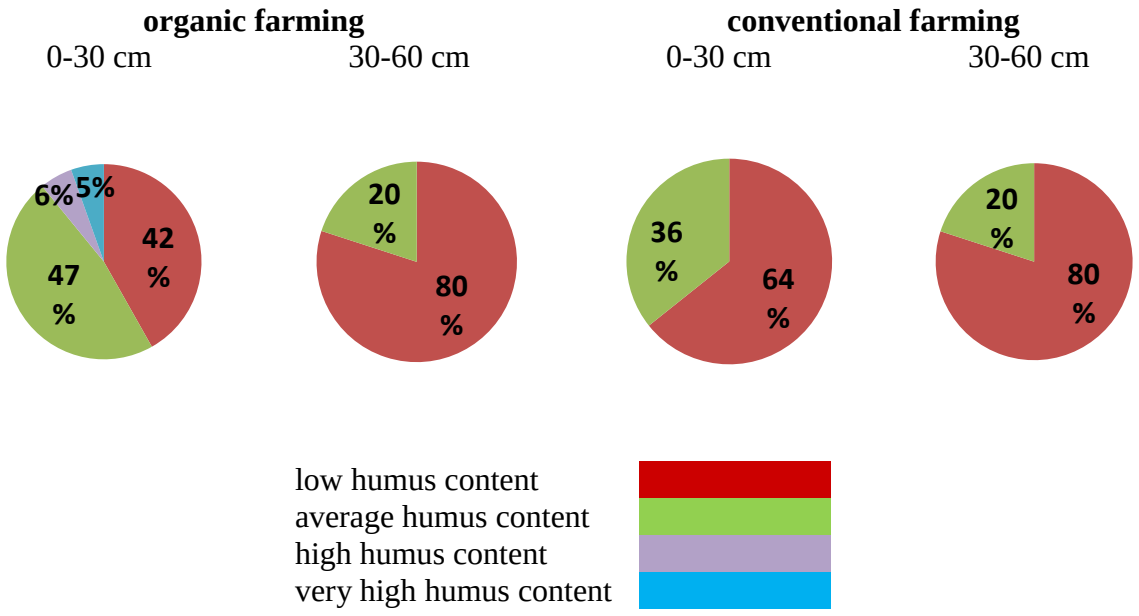


Graph 2 – Mechanical composition of the tested soil (percentage of sand, dust, and clay)

However, concerning that all sampled soils exhibited an equally heavy mechanical composition, it did not make any difference in soil compaction between soils under organic and conventional farming.

At the depth of 10-35 cm, which is the depth of organic manure application, there was a clear difference in soil compaction levels between soils under organic and conventional farming. The lower level of soil compaction in soils under organic farming is caused by application of organic fertilizers, and higher humus content in soils (Graph 3, Table 1).

Sample soils were divided into four groups according to their humus content: low 1-3 %, average 3-5 %, high 5-10 %, and >10 % very high humus content.



Graph 3 – Percentage of sample soils classified by the humus content

Table 1 – Humus content in sampled soils

Farming systems	Depth (cm)	Humus (%)	
		Mean ± SD	Range
Organic	0-30	4,09 ± 2,50	1,88 – 10,81
	30-60	2,23 ± 0,76	1,66 – 3,97
Conventional	0-30	2,92 ± 0,56	2,04 – 3,84
	30-60	2,00 ± 0,65	1,40 – 3,00

Gajić (2006) also reports that soils with higher humus content are more resistant to compaction, due to their elasticity and better structure.

The increase in soil depth by over 60 cm resulted in soils exhibiting similar values of penetrometric resistance (soil compaction) under both farming systems.

Conclusion

The results obtained from testing soil compaction and comparison of results between soils under organic and conventional farming indicate significant differences between these two concepts of farming.

Soils under organic farming exhibited lower values of soil compaction compared to soils under conventional farming, which should be attributed to the use of organic fertilizers (mainly manure). Higher content of humus (organic substances) was found in soils under organic farming. This claim is also supported by the differences observed between soil depth of 10 cm and the depth of organic fertilizer application (about 30-35 cm), while the same levels of soil compaction were observed in deeper soil layers within both farming systems.

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Original scientific paper
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CROP INTERACTIONS IN GREEN BEAN INTERCROPPING WITH LETTUCE AND RADISH

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Abstract

The scope of improving vegetable production through suitable intercrop combinations has not yet been exploited to its full potential. Research goals were to evaluate crop suitability for intercropping systems, to evaluate the effect of fertilization and sowing dates on productivity of green bean, lettuce and radish and to study the main factors responsible for crop performance when intercropped under field conditions in 2009–2010. A field trial was set up in a random block system with four repetitions on experimental field of the Institute for Vegetable Crops in Smederevska Palanka, Serbia. Green bean (*Phaseolus vulgaris* L.) as a main crop was intercropped with leaf lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* var. *radicula* L.). Also, all crops were grown in pure stands. The intercrops were created according to the method of replacement series. The treatments of fertilization consisted of following variants: control treatment without fertilization, microbiological and mineral fertilizers and farm yard manure. All the treatments were examined for two sowing periods, spring and summer.Yield variation was significantly affected by the year of sowing, fertilization and interaction of these factors. LER values were always larger than 1 in intercropping systems. The mechanisms involved in the overyielding were the green bean canopy plasticity and its temporal deployment asynchrony when compared with lettuce and radish, allowing reduction of competition for light and production factors. The study showed that green bean based intercrops might provide the higher total yields.

Keywords*:* *intercropping, green bean, lettuce, radish, LER index*

Introduction

Intercropping is a traditional cultivation practice widely used all over the world (Carruthers et al., 2000; Ghosh et al., 2009). As a type of mixed cropping, it is defined as a cultivation of two or more crops in the same space at the same time (Vandermeer, 1989; Oljača et al., 2000 a).

Legume species are the most common members of intercrops, due to their ability to provide nitrogen through the sybiotic relationship with nitrogen-fixing bacteria. Comparing to corresponding sole crops, higher productivity was approved in many legume based intercrops, such as wheat and chickpea (Betencourt et al., 2012), barley and pea (Launay et al., 2009), maize and peanut (Xiong et al., 2013), maize and soybean (Dolijanović et al., 2013; Oljača et al., 2014) and especially maize and bean (Oljača et al., 2000 a; Mucheru-Muna et al., 2010; Worku 2014). The most of intercropping experiments were related to field crops. However there is lack of data for vegetable intercrops and only a few papers are strictly related to vegetable species (Yildirim and Guvenc 2005; Tosti and Thorup-Kristensen 2010).

Green bean (*Phaseolus vulgaris* L.) is very popular and widely grown vegetable species all over the world (Gepts 1998). As a member of *Fabaceae* family, green bean is in sybiotic relationship with nitrogen-fixing bacteria, and the majority of short green bean varieties mature after 50 to 60 days (Lešić et al., 2004). Lettuce and especially radish are also fast

ripening vegetable species (Lešić et al., 2004). During winter and spring, lettuce and radish are the first fresh vegetables on the market. Green bean, lettuce and radish are mentioned as good or bad neighbours in gardens (Lazić et al., 1991), but there is lack of data related to these intercrops analysed with contemporary scientific methods (Oljača et al., 2000b).
This study was conducted with a purpose to investigate the effects of intercropping green bean with lettuce and radish, different types of fertilizers and different sowing dates on green bean, lettuce and radish yields and productivity of intercrops evaluated using RY and LER indices.

Materials and methods

A two year (2009-2010) field experiment was conducted at the Institute for vegetable crops, Smederevska Palanka (44^o 22' N, 20^o 57'E, altitude 101 m above sea level), in order to evaluete vegetable intercropping systems based on green bean, lettuce and radish. Soil type was vertisol with pH 6.7, 3.13% of organic matter, 0.16% of nitrogen, 0.0% of calcium carbonate, 374.2 ppm of available phosphorus and 335.6 ppm available potasium (Egner et al. 1960).
The experiment was set up as a completely randomized block design with four replications. Lettuce (*Lactuca sativa* L. *cv.* "Neva") and radish (*Raphanus sativus* var. *radicula* L. *cv.* "Non plus ultra") were grown as a sole crops and intercropped with green bean (*Phaseolus vulgaris* L. *cv.* "Palanačka rana"). Green bean was also grown as a sole crop. Four fertilization treatments used were: a) control treatment without fertilization (C), b) microbiological fertilizer (MB), c) mineral fertilizer (NPK) and d) farm yard manure (FYM). All treatments were examined for two sowing periods, spring and summer.
The size of experimental plot was 12,5 m^2, with 0,5 m spacing between different experimental plots. Green bean sole crop experimental plots consisted of 12 rows (0.4 m inter-row spacing) and sowing density was 250 x 10^3 plants per hectare. Lettuce and radish sole crops experimental plots consisted of seedbeds, 1.0 m width, with 0.5 m spacing between seedbeds and sowing densities were 177x10^3 and 758x10^3 plants per hectar, respectively.
For intercropping treatments, the method of replacement series was used (de Wit 1960). Two rows of green bean (0.4) were associated with seedbed of lettuce or radish (0.8 m with). Plant spacing in mixtures was the same as in pure stands of examined crops. The number of plants per unit area in intercrops were 125 x 10^3, 114 x 10^3 and 525 x 10^3 plants per hectare of green bean, lettuce and radish, respectively.
Previous crop was wheat. The common tillage practices, such as plowing and disking were applied during the winter. Before additional tillage, certain experimental plots (fertilization treatments) were fertilized with mineral fertilizer (75 kg of nitrogen, phosphorus and potassium per hectare) or composted farmyard manure (40 t/hectare). Harrow with elastic spikes was used for presowing cultivation. Sowing (lettuce and radish: 13.04.2009. and 13.04.2010; green bean: 14.04.2009. and 16.04.2010.) and weeding were performed manualy.
Plots were watered several times during the growing season. With exception of certain plots mineral fertilizing, all cultivation measures were in accordance with organic production standards.
The microbial fertilizer Slavol (containing: *Bacillus megaterium, Bacillus licheniformis, Bacillus subtilis, Azotobacter chrocoocum, Azotobacter vinelandi, Derxia* sp.) was applied during the first true leaf stage of green beans. The application was performed using 1,5% microbial fertilizer aqueous solution in the amount of 6 l/ha. The treatment was repeated after 10 days.
At radish, lettuce and green bean maturity stage, iner rows were harvested and used to calculate yields per hectare, Relative yields (RY) and Land equivalent ratio (LER). LER is sum of the relative yields (RY) of intercrops (Vandermeer, 1989), green bean with lettuce

(LER = $RY_{green\ bean}$ + $RY_{lettuce}$) and green bean with radish (LER = $RY_{green\ bean}$ + RY_{radish}). The relative yields of green bean, lettuce and radish were calculated by following equation: RY = I / M (I is yield of some crop per hectare in intercrop and M is its yield per hectar in monoculture).

Results and discussion

Average monthly temperatures and rainfalls during the two experimental years, are shown in Figure 1. Comparing to long-term average, both experimental years were characterized by higher temperature values and rainfalls. In 2009 annual temperature mean (12,42°C), and average monthly temperatures (except november) were significantly higher than long term temperature means for Smederevska Palanka. Annual rainfall sum was 788 mm, but in april and may, extreme drought, uncommon for spring time, was noticed. Another drought period with less rainfalls occured in august and september. In 2010, similarly as the previos year of trial, annual temperature mean (12,6°C), and average monthly temperatures (except october and november) were significantly higher than long term temperature means. Despite the higher annual rainfall sum (730 mm), drought period appeared in august.

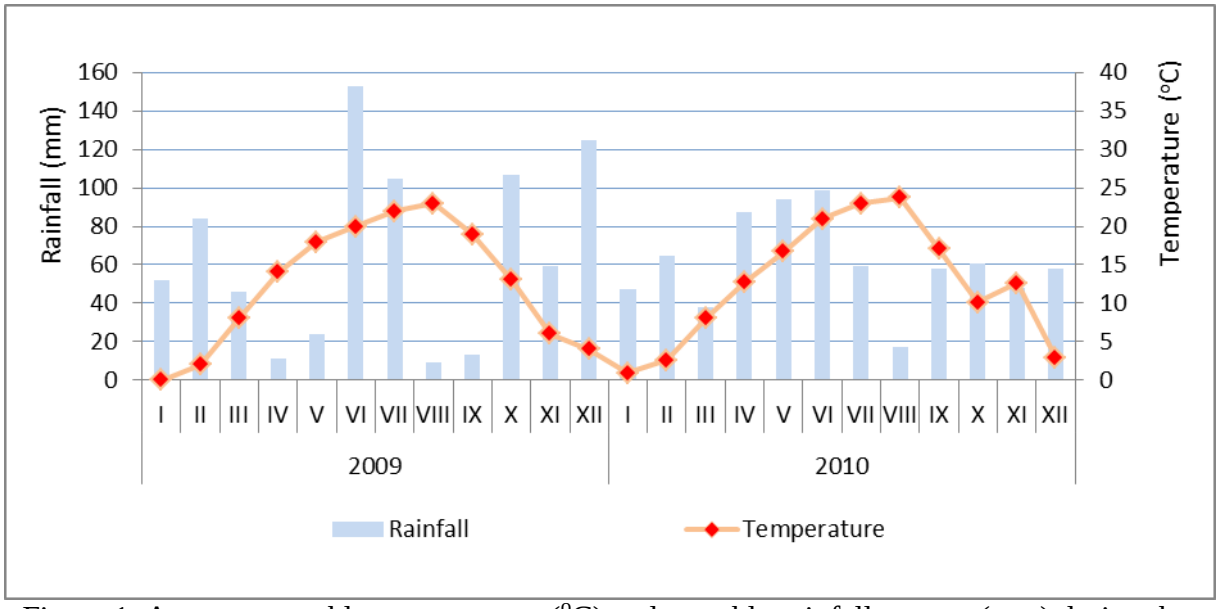

Figure 1. Average monthly temperatures (°C) and monthly rainfall summs (mm) during the two experimental years (2009-2010)

Green bean, lettuce and radish yields were affected by tested fertilizers, as shown in Table 1. Comparing to control treatment, higher green bean, lettuce and radish yields were recorded on the plots treated with microbiological fertilizer and farmyard manure. Similar results were already reported for green bean (Stone et al., 2003) and lettuce yields (Okur et al., 2008). Radish yield was also affected by tested fertilizers but, in this trial it was lower than yield reported by Miladinović et al. (1997). The highest green bean, lettuce and radish yields were recorded on treatments with the mineral fertilizer, probably due to a high availability of major nutrients.

Table 1. Yields of sole crops (SC), relative yields (RY), standard errors (SE) and land equivalent ratio (LER) of intercropped (IC) green bean with lettuce and radish, treated with different fertilizers, during the two growing seasons (two years average: 2009-2010)

IC	Treatment	SC yield	SE	RY	SE	LER	SE

First sowing date (spring)							
Green bean	Control	9,17	0,194	0,54	0,014	1,2	0,014
Lettuce		15,14	0,715	0,66	0,013		
Green bean	MB	10,04	0,149	0,52	0,01	1,17	0,014
Lettuce		17,84	1,158	0,65	0,008		
Green bean	NPK	10,47	0,132	0,53	0,006	1,2	0,005
Lettuce		20,53	0,812	0,67	0,008		
Green bean	FYM	9,92	0,197	0,52	0,012	1,17	0,018
Lettuce		17,94	0,77	0,65	0,01		
Green bean	Control	9,17	0,194	0,54	0,012	1,23	0,015
Radish		5,03	0,228	0,69	0,011		
Green bean	MB	10,04	0,149	0,51	0,009	1,2	0,01
Radish		5,45	0,265	0,69	0,009		
Green bean	NPK	10,47	0,132	0,51	0,008	1,2	0,008
Radish		5,86	0,119	0,69	0,004		
Green bean	FYM	9,92	0,197	0,5	0,017	1,21	0,031
Radish		5,66	0,19	0,71	0,017		
Second sowing date (summer)							
Green bean	Control	9,99	0,154	0,49	0,013	1,16	0,014
Lettuce		13,8*	0,215	0,66	0,01		
Green bean	MB	10,23	0,093	0,51	0,009	1,17	0,004
Lettuce		14,91*	0,522	0,66	0,009		
Green bean	NPK	11,33	0,13	0,51	0,018	1,17	0,027
Lettuce		16,45*	0,338	0,66	0,017		
Green bean	FYM	10,3	0,236	0,51	0,015	1,2	0,027
Lettuce		15,72*	0,495	0,69	0,012		
Green bean	Control	9,99	0,154	0,5	0,014	1,21	0,017
Radish		6,58*	0,311	0,71	0,008		
Green bean	MB	10,23	0,093	0,48	0,013	1,19	0,009
Radish		6,86*	0,107	0,71	0,011		
Green bean	NPK	11,33	0,13	0,5	0,005	1,2	0,011
Radish		7,33*	0,304	0,7	0,007		
Green bean	FYM	10,3	0,236	0,52	0,019	1,25	0,016
Radish		7,45*	0,231	0,72	0,006		

Control – without fertilizer; MB – microbiological fertilizer; NPK – mineral fertilizer (15:15:15); FYM – highly decomposed farmyard manure; * - blossom emergence;

Subjected to the sowing season, green bean and radish yields were higher in the second sowing date but lettuce yields were higher in the first sowing date. That was not in accordance with results of Ferreira et al. (2006) who reported lower green bean yields in later sowing periods. Also, in second sowing period, green bean treated with FYM, achieved higher yields than those treated with microbiological fertilizer. It was presumably caused by better soil properties and water accumulation, improved with high amounts of organic matter, contained in FYM. However, in second sowing period, in lettuce and radish sole crops and intercrops, regardless to fertilizing treatments, frequent blossom emergence was noticed. High temperatures and long day terms could promote blossom emergence (Miladinović et al. 1997), so the lettuce and radish yield quality was significantly reduced.

Green bean relative yields (RY) varied about 0,5 in both intercrops, i.e. relative yields of green bean were not significantly affected by intercropping, regardless of another intercropped species. Slightly higher green bean RY values were recorded in the first sowing

season but it is not definetly clear what caused these differences. Lettuce and radish RY were above 0,5 and influenced LER index values higher than one. LER is usually used for an intercrop efficacy evaluation. LER index higher than 1, sugests that the intercropping is much more efficient than sole crops and that the competition for light, water and soil resources among intercropped species is not significant (Vandermeer 1989).
Oppositly to first sowing period, in the second sowing period, LER index values of intercrops treated with FYM were higher than untreated or those treated with other fertilizers. It is probably corelated with soil water availability during the drought period. Hati et al. (2006) reported better rooting of soybean and improved physical properties of the soil after farmyard manure application.

Conclusion

Examined intercrops approved as a better choice, comparing to related sole crops. They could be useful in achieving higher total yields, regardless to applied fertilizer or sowing date. However different lettuce and radish genotypes should be used because the tested genotypes are not suitable for late season production. Application of microbiological fertilizers and highly decomposed farmyard manure are suitable for organic green bean/lettuce or green bean/radish intercropping production and also could lead to yield enhancing.

Acknowledgement

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THE IMPACT OF BIOCHAR ON HYDRAULIC CONDUCTIVITY OF THE SOIL

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Abstract

Biochar as a highly porous material, thus its application to soil is thought to improve a range of soil hydro-physical properties. However, compared to these properties, little is known about the impact of biochar on soil hydraulic conductivity. The aim of this study was to evaluate the effect of biochar on soil hydraulic conductivity of sandy loam soil. Research study was undertaken at experimental site of Slovak University of Agriculture in Nitra (locality Malanta, near Nitra). Nine plots were set out on the experimental area. The field experiment consisted of 3 levels of biochar application 0, 10 and 20 tha^{-1}. Biochar was applied in March 2014 into the depth 0 – 10 cm of soil in 3 replications. Samples of soil were taken 53 days after applications of biochar. Saturated soil hydraulic conductivity was measured using Falling Head Method (FHM), based on measurement with variable hydraulic slop. After 53 days biochar amendment improved soil hydraulic properties. The rates of biochar significantly increased soil hydraulic conductivity.

Keywords: *biochar, saturated hydraulic conductivity,*

Introduction

Carbon plays very important role in soil. It has the potential to influence physical, hydro-physical and chemical processes in soil (DeLuca et al., 2006; Glaser et al., 2001). It has also been shown to affect soil productivity, quality and fertility and nutrient cycling, which all affect crop production (Skjemstad et al., 2002; Lal, 2004). The understanding how the addition of carbon may influence the soil is essential. The products produced from pyrolysis include a gaseous material referred to as ‘‘syngas’’ and a carbon (C) rich, charcoal material known as biochar (Lehmann, 2007).

Biochar is a stable, recalcitrant organic carbon material created by pyrolysis of biomass at temperatures between 300 and 1000°C under low or no oxygen conditions (Jeffery et al. 2011; Krull 2011; Verheijenet al.2010).

The application of biochar made to the soil has the potential to reduce the need for nitrogen fertilizer, as well as enhance soil chemical and physical properties (Krishnakumar et al, 2013). Biochar is highly porous, thus its application to soil is considered to improve arrange of soil physical properties including; total porosity, pore-size distribution, soil density, soil moisture content, water holding capacity or plantavabter content (PAWC) and hydraulic conductivity (Atkinson et al. 2010; Major et al. 2009; Sohi et al.2010; Sohi et al. 2009b; Zwieten et al. 2012). The possible mechanisms by which coal-derived humic acids improve soil physical properties are the formation of organic-mineral complexes by functional groups of the humic acids. The hydrophobic polyaromatic backbone reduces the entry of water into the aggregate pores leading to an increased aggregate stability and water availability.

Given the importance of hydraulic conductivity in determining the partitioning of precipitation between infiltration and overland flow (Mishera et al. 2003), which impacts water storage in the subsurface and thus plant available water, it is necessary to understand the effects of biochar on the hydraulic properties of different soil types.

Deveraux et al. (2012) estimate that biochar significantly decreased saturated hydraulic conductivity, on the other hand biochars produced from the pyrolysis of corn stover feedstock

at 350 and 550 °C temperatures significantly increased K of the Typical Fragiaqualf soil (Herath et al. 2013). Biochar amendment decreased saturated hydraulic conductivity by 92% in sand and 67% in organic soil, but increased K by 328% in clay-rich soil (Barnes et al. 2014).
This manuscript provides detailed assessment of the effects of applying 10 and 20 t/ha paper fiber sludge and grain husks biochar to a sandy loam soil in Nitra region of Slovakia.

Materials and methods

Experimental area

This field trial was conducted in March 2014 at the experimental site of SAU-Nitra (Nitra-Malanta) in Nitra region of Slovakia (lat. 48°19′00′′; lon. 18°09′00′′). The soil type is classified as OrthicLuvisol (FAO, 1998). The average annual air temperature was 10.3°C and annual precipitation was 640mm during the studied year (2014).

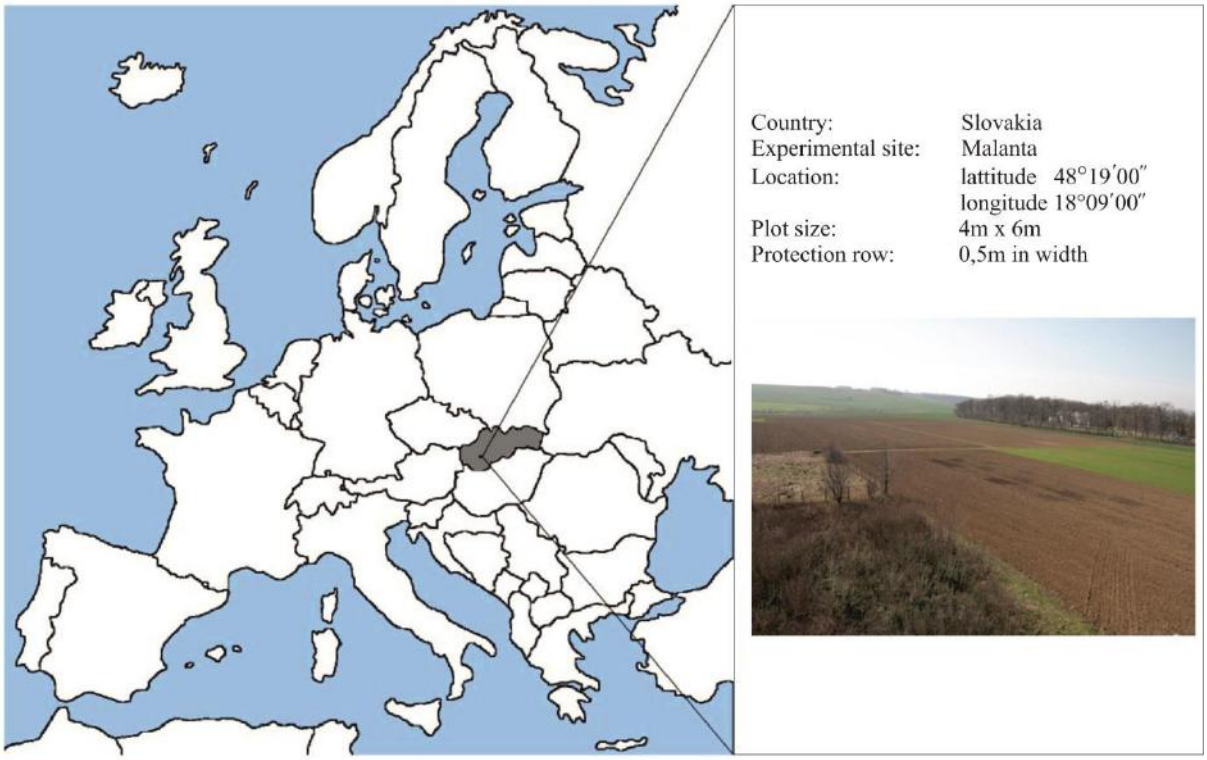

Fig. 1 Experimental site of SAU-Nitra (Nitra-Malanta) in Nitra region of Slovakia (lat. 48°19′00′′; lon. 18°09′00′′)

The field experiment consisted of treatments: Control (without adding biochar), B10 (soil+biochar in rate 10 t/ha) and B20 (soil+biochar in rate 10 t/ha). Every plot was in 3 replications. There were 9 plots (4m x 6m), separated by a protection row 0.5 m. Biochar was applying into the soil in March 2014 and 53 days after application there were taken the samples of soil for measuring of satured hydraulic conductivity.

Biochar characterization

Biochar used for the field experiment was produced from paper fiber sludge and grain husks (1:1 w/w) (company Sonnenerde, Austria) by pyrolysis at 550°C for 30 minutes in a Pyreg reactor (Pyreg GmbH, Dörth, Germany).

Tab. 1 Characteristics of biochar

	C / %	C / g kg^{-1}	N / %	N / g kg^{-1}	H / %	H / g kg^{-1}	O / %	O / g kg^{-1}
Biochar	53.1	531	1.4	14	1.84	18,4	5.3	53

Determination of hydraulic conductivity using falling head method

There was used device working on principle of measurement with variable hydraulic slope (i.e. above the measured soil sample changes the water level during time) to determine the hydraulic conductivity in laboratory condition.

Soil samples were taken into non-corrodible metal rollers with volume of 100 cm^3. It is necessary to have soil moisture at the value close to field capacity for taking undisturbed soil samples so therefore the sampling date was 2 days after intensive precipitations. Soil sample roller was lightly pressed into to soil using lever lifter. Soil sample was saturated by water before taking a measurement. The saturation lasted for 24 hours. The value of hydraulic conductivity coefficient was calculated according to equation:

$$K = \frac{L}{t}.\ln\frac{H_2}{H_1} \qquad (cm.s^{-1})$$

where:

L is the length of roller (cm), t is time of water drop from height H2 to H1(s), H_2 is an initial height of water in the extension piece, it is another roller whose flow surface is equal to flow surface of soil sample (cm), H_1 is the height of water in the extension piece after drop of water level (cm).

Statistical analysis

The statistical processing of the data included the determination of means and standard deviations. One-way analysis of variance (ANNOVA) at $p<0.05$ was performed to examine the effect of biochar at different application rates on satured hydraulic conductivity. Significant differences between means of three replications were identified using a least significant difference (LSD at $p<0.05$) test using STATGRAPHICS Centurion statistical software.

Results and discussion

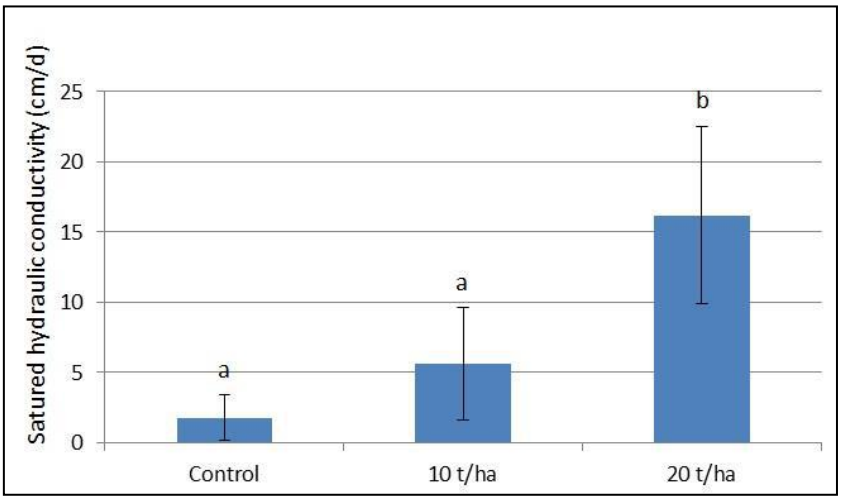

Fig. 2 Satured hydraulic conductivity 53 days after application of biochar into the soil in depth 0-5cm (± standard deviation, n=3). Different letters indicate significant difference between treatments means by the LSD test ($p < 0.05$).

Fig. 1 presents saturated hydraulic conductivity of loamy soil 53 days after application of biochar on field experiment at the experimental site of SAU-Nitra (Nitra-Malanta) in Nitra region of Slovakia. Biochar was applied in rates 10 and 20 t/ha. The average of control was 1,784 $cm.d^{-1}$. Figure shows that the boths rates of biochar increased soil hydraulic conductivity. However, there was not a statistically significant difference between the control and rate of biochar 10 t/ha ($p < 0.05$). The application 20 t/ha of biochar into the soil significantly increased satureded hydraulic conductivity by 906,22 % ($p < 0.05$). It was from 1,784 to 16,167 cm/d. The same situation was in publication of Barnes et al. (2014), there was increased satured hydraulic conductivity by 328% in clay-rich soil.

Conclusion

We used in the experiment 2 rates of biochar 10 and 20 t/ha, which were applicated into the soil. Saturated hydraulic conductivity was measured 53 days after application using falling head method . There was not a statistically significant difference between the control and rate of biochar 10 t/ha ($p < 0.05$). The application 20 t/ha of biochar into the soil significantly increased saturated hydraulic conductivity. Our study demonstrated that biochar has effect on satured hydraulic conductivity. It is very important the rate of biochar and biomass for produce bichar and also the type of soil. We demonstrated that 20 t/ha of biochar produced from paper fiber sludge and grain husks applicated to the loamy sand soil increased satured hydraulic conductivity.

Acknowledgement

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Original scientific paper
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POTENTIAL OF RHIZOBIUM AND BRADYRHIZOBIUM SPECIES AS PLANT GROWTH PROMOTING RHIZOBACTERIA ON PADDY (Oryza sativa L.)

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Abstract

Rice is the staple food in Sri Lanka. Due to rapid growth of population, the production of rice also needs to be increased rapidly. The search of microbial inoculants that improve soil fertility and enhance plant nutrition has continued to increase, due to the negative health and environmental impacts of using synthetic fertilizers. A greenhouse experiment was established in Mihintale, dryzone of Sri Lanka, to evaluate the effects of inoculation of two traditional paddy varieties (Kaluheenati and Suwandel) and one improved paddy variety (Bg 300) with different *Rhizobium* spp. on the growth and yield. *Rhizobium* strains isolated from the nodules of *Glycine max*, *Mimosa pudica*, *Vigna radiata*, *Psophocarpus tetragonolobus*, *Vigna unguiculata*, *Vigna unguiculata* subsp. *sesquipedalis*, *Clitoria ternatea* and *Sesbania grandiflora* were tested for their potential to promote growth and yield of rice grown in potted soils with the un-inoculated control and had five replicates. Synthetic fertilizer was not added.

Some *Rhizobium* spp. inoculations increased relative growth rates and yield of paddy plants significantly ($p<0.05$) over un-inoculated controls in each of three paddy varieties. Further, significantly higher ($p<0.05$) performance was observed with the inoculation of *Rhizobium* spp. isolated from *Sesbania grandiflora*. Therefore, we conclude that *Rhizobium* spp. inoculants can be used to promote growth and yield even for the improved variety of paddy Bg 300. *Rhizobium* spp. inoculants can substitute the synthetic fertilizers even in improved paddy variety which earlier thought as highly synthetic fertilizer dependent. Therefore, the use of microbial inoculants should be maximized for enhance paddy crop production to meet the demands of an increasing human population and enables to supply healthy diet to human and increasing the number of healthy life years without any chronic disease.

Key words: *Rhizobium* spp., microbial inoculants, traditional paddy varieties, improved paddy varieties, synthetic fertilizer

Introduction

Rice (*Oryza sativa*) is the staple food of Asia and part of the Pacific. In ancient times, before the introduction of hybrid rice varieties, over 400 different varieties were grown all over the Sri Lanka. After the Green Revolution in the late 1960s, development of high-yielding varieties of cereal grains, expansion of irrigation infrastructure, modernization of management techniques, distribution of hybridized seeds, synthetic fertilizers and pesticides to farmers were introduced (Hazell and Peter, 2009).

It is estimated that there will be about 4.8 billion people will need to be fed with 760 million tons of rice in year 2020 (Hussain *et al*., 2009). Therefore, the production of rice needs to be increased by 2 percent per year to meet future demands (Hussain *et al*., 2009). Sri Lankan agricultural sector mainly base on paddy cultivation. However, most of the farmers in Sri Lanka still practicing conventional agriculture with using chemical fertilizers and pesticides. Hence there is severe need to develop agriculture practices, which can fulfill crop production and protection requirements economically on sustainable basis.

Use of sustainable or organic agriculture can reduce most of negative effects attributed from conventional agriculture and has potential benefits in enhancing soil quality (Maeder *et al*., 2002). Many types of microorganisms have ability to build strong interactions with the plants, being a member of plant rhizosphere. *Rhizobia* are soil bacteria that fix atmospheric nitrogen after becoming established inside root nodules of specific host legumes. Despite being unable to induce nodulation, rhizobia have been shown to be able to infect and colonize the roots of paddy forming root endophytic interactions which can act as plant growth promoting rhizobacteria (PGPR). They enhance PGP activities like mobilization and efficient uptake of nutrients (Biswas *et al*., 2000b), enhancement in stress resistance (Mayak *et al*., 2004), solubilization of insoluble phosphates (Hussain *et al*., 2009), produce plant growth hormones (Dakora, 2003) and siderophores (Hussain *et al*., 2009).
Few researches were conducted to see effect of *Rhizobium* spp. in growth of paddy (Yanni *et al*., 1997). But there is little or no evidence about traditional varieties with endophytic *Rhizobiu*m spp. Because most of the studies conducted to hybrid, fertilizer dependent rice varieties. As such this study was carried out with the objective of finding the rhizobial strains which promote growth and yield of some selected traditional and improved paddy varieties in Sri Lanka.

Materials and methods

A pot experiment was conducted on July to November, 2014 in the greenhouse at the Rajarata University, Mihinthale, Sri Lanka (8° 18′ 44N latitude and 80° 24′ 47E longitude).The temperature during the study period was 29.0-35.0 °C. Annual precipitation is between 1000-1500 mm and receives inter monsoon and the North-East monsoon rains mostly.

Paddy varieties

Seeds of two traditional paddy varieties of Sri Lanka called as “Kaluheenati” (KH), “Suwendel” (SD) and one improved rice variety as “Bg 300” (BG) were obtained from the Rice Research Center, Department of Agriculture in Batalagoda, Sri Lanka.

Isolation and inoculum preparation of *Rhizobium* and *Bradyrhizobium* strains

Different *Rhizobium* strains were isolated from the surface sterilized fresh nodules of *Glycine max* (T_1), *Mimosa pudica* (T_2), *Vigna radiata* (T_3), *Psophocarpus tetragonolobus* (T_4), *Vigna unguiculata* (T_5), *Vigna unguiculata* subsp. *sesquipedalis* (T_6), *Clitoria ternatea* (T_7) and *Sesbania grandiflora* (T_8) on yeast extract mannitol (YEM) agar. Pure cultures of the isolated strains were grown for 72 h to a density of 10^8- 10^9 CFU/ml in yeast extract mannitol (YEM) broth with shaking at 80 rpm and 30°C (Hussain *et al*., 2009) .

Pot experiment design and *Rhizobium* and *Bradyrhizobium* strains inoculation

The 3 kg of surface soils (0-15 cm in depth) of the field in Mihinthale, Sri Lanka was dispensed into each 108 pots. The soil was clay loam with 6.91 pH, 46 µS/m electrical conductivity and 67% moisture content. Water content was set to 3 cm high from the soil surface. There were eight treatments of *Rhizobium* or *Bradyrhizobium* were inoculated and one un-inoculated control. The experiment design was a Randomized Complete Block Design (RCBD) with three rice varieties and four replicates and one plant per pot.

Seedlings of three days age were gently uprooted from the seed bed and were treated with 2 ml of freshly prepared different rhizobial strains in YEM broth (10^8-10^9 CFU/ml) accordingly and were transplanted to the pots. Pots were kept under natural light conditions and watered regularly to maintain 3 cm high water level. Weeding was done manually and never used any

chemical pesticide or weedicide to manage pest and weed attacks. Ash was sprinkled over paddy plants as a repellent to pests and some of the insect repellent plants were grown inside the greenhouse.

Growth and yield parameters

Growth data such as plant height, number of tillers and number of leaves, length and width of the leaves were recorded in one month intervals. Yield data such as number of panicles, grains per panicle and un-filled (chaff) grains per panicle and dry weight of 100 grains were recorded.

Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) to compare growth and yield of paddy with different rhizobial treatments and paddy varieties. Means were compared by using the Tukey's Studentized Range Test (SAS version 6.12) at a significance level of $p<0.05$ (SAS Institute, 1996).

Results and discussion

Growth and yield parameters

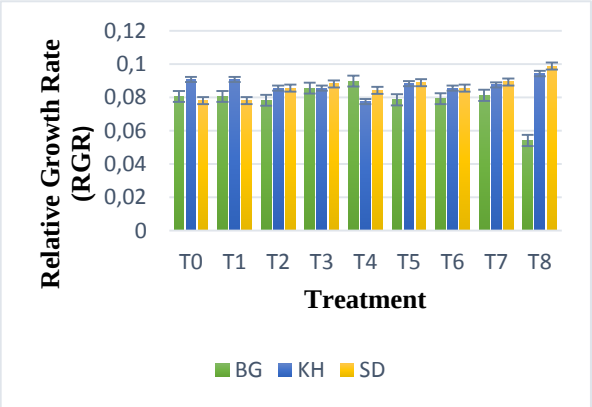

Fig.1. Effect of *Rhizobium* inoculation on relative growth rate (RGR) of selected three rice varieties under pot conditions. Error bars indicate 95%

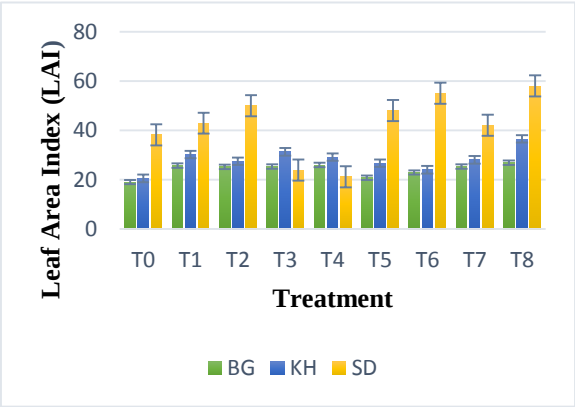

Fig.2. Effect of *Rhizobium* inoculation on leaf area index (LAI) of selected three rice varieties under pot conditions. Error bars indicate 95% confidence

The statistically significant increase ($p<0.05$) in relative growth rate (RGR) was shown *Rhizobium* strain isolated from *Sesbania grandiflora* (Fig.1). However, there was no significant increase shown with the other inoculated rhizobial species isolated from the other legume plants (T_1- T_7) over un-inoculated control (T_0). Also there was no significant increase ($p= 0.1088$) in RGR when considering the variety alone (Fig. 1). The increase in RGR might be the result of production of indole acetic acid and gibberellins when the effective endophytic association occurred (Singh *et al*., 2005).

There was statistically significant increase ($p= 0.0001$) shown in leaf area index of ‘Suwadel’ (SD) paddy variety with particular rhizobial strain inoculation compared to other two varieties (Fig. 2). Treatment comparison also showed significant difference ($p=.0015$) of LAI and the rhizobial strain isolated from *Sesbania grandiflora* (T_8) showed the highest response.

Significant variety and treatment effect ($p=0.0015$) was observed in number of leaves (Fig.3). Paddy varietal difference also significant ($p=0.0001$). Effect of T_8 treatment on SD variety was significantly prominent. Hussain *et al*., (2009) published results indicated that *Rhizobium* spp. isolated from green gram shown significantly high leaf numbers than those isolated from chick pea. Green gram has *Rhizobium phaseoli* and chick pea has *Mesorhizobium* sp. (Hussain

et al., 2009). These findings were also similar to the results of the present study which was observed that variation in relative efficiency of growth parameters of different paddy varieties with particular rhizobial strains can be explained by their ability to cope with specific paddy variety.

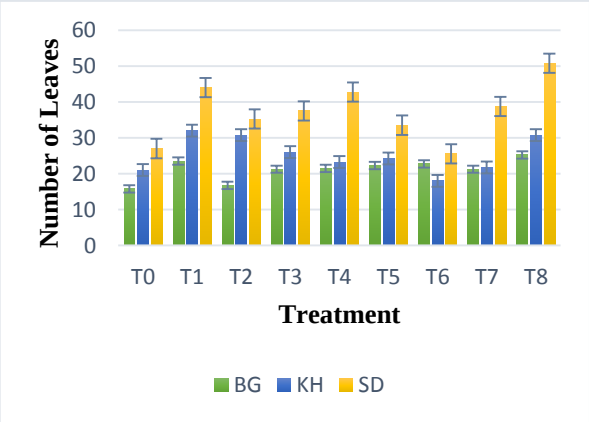

Fig.3. Effect of *Rhizobium* inoculation on number of leaves of selected three rice varieties under pot conditions. Error bars indicate 95% confidence

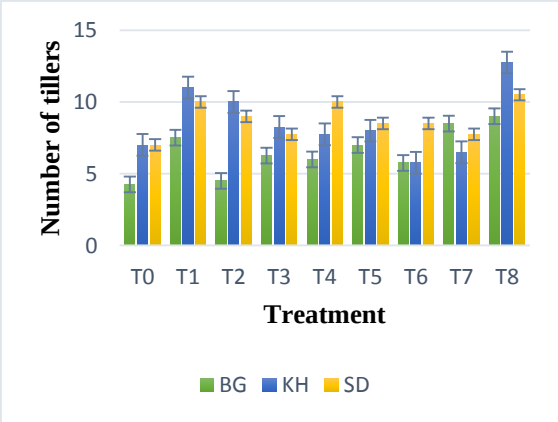

Fig.4. Effect of *Rhizobium* inoculation on number of tillers of selected three rice varieties under pot conditions. Error bars indicate 95%

Tiller numbers were shown significance difference (p=0.016) in variety and treatment interactions (Fig. 4). There was a significant difference in tiller numbers with the paddy variety (p=0.0001). Considering overall treatment effect the paddy variety Bg 300 showed lower number of tillers with the different rhizobial strains.

Panicle numbers were changed significantly when considering the variety and treatment interaction (p=0.016). Further it was shown that T_8 increase the number of panicles in all three varieties significantly over un-inoculated control (T_0) (Fig. 5). There were significant varietal difference also observed in panicle numbers and ‘Kaluheenati’ (KH) variety got the highest number of panicles despite the inoculated rhizobial strain (p= 0.0001). These results were in lined with Biswas *et al.*, (2000b) who have reported 16% increase in number of panicles in paddy plant and suggested that the improvement due to increased availability of nutrients and phyto-hormones such as indole acetic acid and ethylene. Similarly Peng *et al.*, (2002) concluded at increment of 27.11% in number of panicles per plant of rice due to *Rhizobium* inoculation over un-inoculated control. They were suggested efficient nutrient and water uptake as important mechanisms for that. Furthermore, differential behavior of different PGPR strains of rhizobia against common host has also been reported by Piesterse *et al.*, (2001).

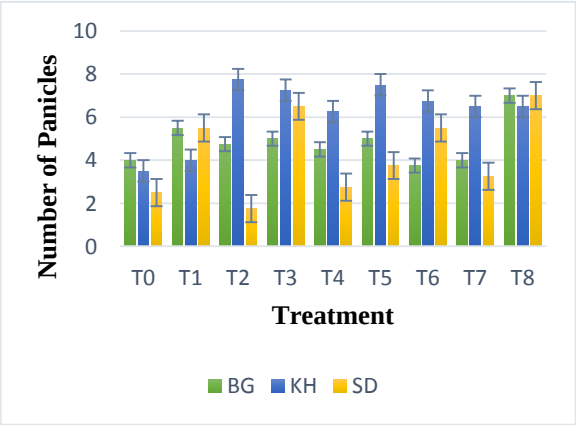

Fig.5. Effect of *Rhizobium* inoculation on number of panicles of selected three rice varieties under pot conditions. Error bars indicate 95%

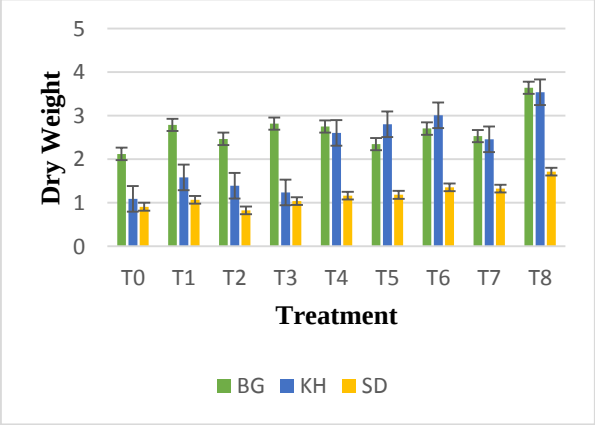

Fig.6. Effect of *Rhizobium* inoculation on dry weight of hundred grains of selected three rice varieties under pot conditions. Error bars indicate 95%

It was observed significantly different variety and treatment combination statistically on dry weights of 100 grains in all the rhizobial inoculation treatments (p=0.016). Again the specific rhizobial strain isolated from *Sesbania grandiflora* (T_8) showed highest dry weights of 100 grains in all three paddy varieties (Fig. 6). Similarly Hussain *et al.*, (2009) showed that maximum significant increase of 36.74% in the thousand grain weights of *Rhizobium* spp. inoculated paddy in comparison to un-inoculated control.

Conclusion

Specific *Rhizobium* spp. isolated from *Sesbania grandiflora* and *Glycine max* could improve paddy growth and yield. Growth and yield increase of two traditional and improved paddy varieties depend on the enhancing PGPR activity of the specific rhizobial strains. Even high inputs demanding, improved paddy variety also positively responds to the specific *Rhizobium* spp. inoculation. These results establish a strategy of using selected rhizobial strains to promote rice production capacity in sustainable agriculture practices.

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Original scientific paper
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CAN BIOCHAR AMENDMENT BE AN ECOLOGICAL FARMING TECHNOLOGY TO INCREASE SOYBEAN (Glycine max L.) GROWTH AND YIELD?

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Abstract

The agriculture sector is the foundation in Sri Lanka's economy. Most of the farmers in Sri Lanka tend to use chemical fertilizers for their cultivation processes. Synthetic fertilizers cause depletion of the nutritional content of foods and exposure to chemical fertilizers cause serious health problems. Therefore, the use of organic amendments can be a reliable solution to avoid many threats to the life caused by the application of chemical fertilizers. Biochar is a type of soil amendment helps in improving the quality of environment and human health. This research was conducted to find the effectiveness of biochar as an alternative to chemical fertilizer. For this purpose, soybean (*Glycine max* L.) was grown under tropical conditions in Mihintale, Sri Lanka, on red yellow podzolic soils in the greenhouse in ten-fold replication. Treatments were comprised control, wood biochar (15% by weight), saw dust biochar (15% by weight), compost (15% by weight),synthetic chemical fertilizer (111.5kg Urea ha^{-1}, 111.5kg P_2O_5 ha^{-1}, and 82.9kg K_2O ha^{-1}) and different combinations of above amendments together. The wood biochar treatment was found to significantly increase the plant height, number of leaves, number of nodules and number of pods in comparison to no biochar treatments. We conclude that biochar is promising to potentially improve growth and yield of soybean. The basic concept of biochar production is simple and therefore, it can be easily produced locally by farmers. Therefore, farmers can use wood biochar in the cultivation of soybean in sustainable agriculture practices to minimize the negative health effects caused by chemical fertilizers.

Keywords: *Wood biochar, saw dust biochar, organic supplement, compost, synthetic chemical fertilizer*

Introduction

Utilization of organic fertilizers can be a reliable solution to avoid soil pollution and many other threats to the environment and life caused by overuse of chemical fertilizers. The sustainable agricultural methods do not use synthetic fertilizers and pesticides. It attempts to close nutrient cycle on the farms, protect the environment quality and enhance beneficial biological interactions and processes. Many researchers found that comparing to conventional agriculture, organic crops yielded 95 – 100 % percent (Major *et al*., 2005),along with 50% lower expenditure on fertilizer and energy as well as 97% less pesticides (Mader, 2002).

Biochar is a kind of soil amendment. It is a type of charcoal prepared from the pyrolysis of a range of biomass feedstock like wood, crop, yard wastes and manures (Novak *et al*., 2009). The primary function for biocharis as a soil enhancement to facilitate retention of water and nutrients and a habitat for soil microbes (Baldock and Smernik, 2002). Biochar usage has been revealed to promote soil nutrient quality and stabilize soil than other organic amendments (Lehmann and Joseph, 2009). Laboratory studies estimated that biochar has a residence time in soils on the order of 1300–4000 years (Cheng *et al*., 2008). Research reveals that beneficial mycorrhizal relationships and biological nitrogen fixation together are improved by biochar applications in common beans (*Phaseolus vulgaris*) (Rondon*et al*., 2006). Researches have also shown that the biochar characteristics important to plant growth can be improved with time after its incorporation into soil (Major *et al*., 2010).

Soybean (*Glycine max*) which is one of the major and widely cultivated legume crops in dryzone of Sri Lanka. Application of innovative agricultural method to improve the growth and yield of this crop against the continuing depletion of soil organic nutrients will help in improving the livelihood of this rural population. It was therefore, the aim of this research to investigate the effect of biochar on the growth and yield of soybean in dry zone of Sri Lanka.

Materials and Methods

Study site

The study was conducted under greenhouse conditions and natural light at the Rajarata University of Sri Lanka in Anuradapura district, dry zone of Sri Lanka(8° 21' 0" North, 80° 30' 0" East) on red yellow podzolic soils from July 2014 to December 2014. The environmental temperature during the trial was 29.0-35.0 °C. Annual precipitation is between 1000-1500 mm and receives inter monsoon and the North- East monsoon rains mostly.

Biochar production

The biochar use in this study was produced from wood chips and saw dust. Dried wood chips of *Cinnamomumzeylanicum, Mangiferaindica, Micheliachampaca, Heveabrasiliensis, Anacardiumoccidentale*used for the production of wood biochar. Fresh woods of *Artocarpusheterophyllus, Meliadubia, Tectonagrandis, Swieteniamahagoni* used for the production of saw dust biochar. Biochar was produced at a 450 -550 °C pyrolysis temperature using the two-barrel nested design. Organic matter used to produce biochar was filled in to the small barrel and the lid was closed. The small barrel was placed in the inverted position inside the large barrel. The organic matter in the inner barrel was heated to about 450 – 550 ° C by burning wood scraps stuffed into the space between the inner and outer barrels. The produced biochar was ground by passing through a 2 mm sieve.

Experimental design and data collection

A randomized complete block design (RCBD) was used with ten replicates in this experiment. The experiment included control and six treatments as, control without amendments (T_1), field soil + wood biochar (15% by weight) (T_2), field soil + saw dust biochar (15% by weight) (T_3), field soil + compost (15% by weight) (T_4), field soil + synthetic chemical fertilizer (111.5 kg N ha^{-1}, 111.5 kg P ha^{-1}, and 82.9 kg K ha^{-1}) (T_5), field soil +wood biochar (15% by weight) + compost (15% by weight) (T_6), field soil + saw dust biochar (15% by weight) + compost (15 % by weight) (T_7). Soils were sampled in the field in Anuradhapura, Sri Lanka, at depth 0- 15 cm, air dried and then ground to pass a 2 mm sieve and prepared for a total weight of 3 kg pot^{-1} and mixed with various amendments accordingly. *Gliricediasepium*leaves were air dried and 100 g of ground leaves were added to each pot. Data were subjected to analysis of variance (ANOVA) and mean separation using the Tukey'sStudentized Range Test (SAS version 6.12) at a significance level of $p<0.05$ (SAS Institute, 1996).

Results and discussion

Potential growth and yield of soybean

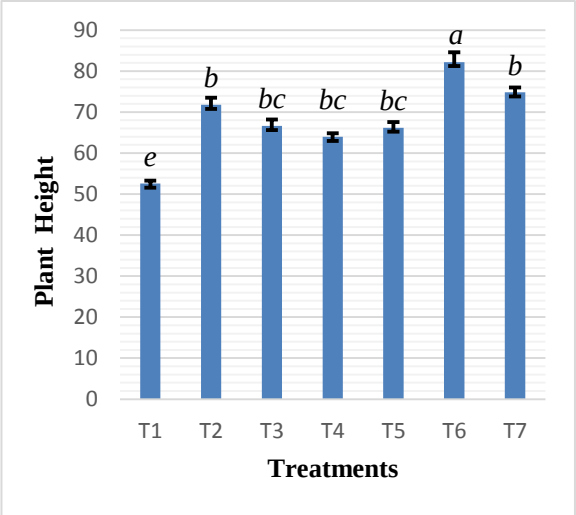

Fig.1.Plant height of soybean plant at different biochar,organic and fertilizer amendments. Means denoted with different letters are significantly different at p< 0.05.

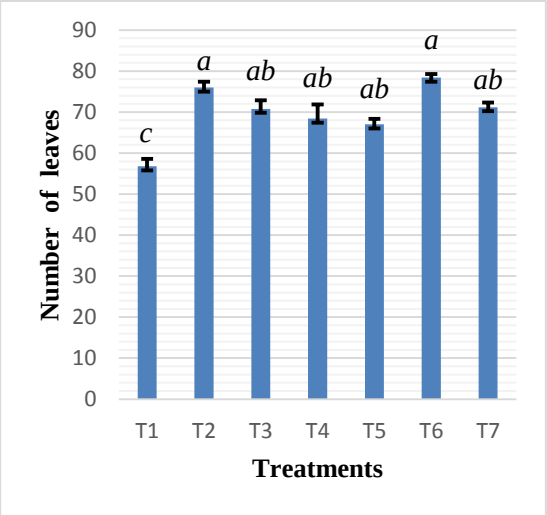

Fig. 2.Number of leaves of soybean plant at different biochar, organic and fertilizer amendments. Means denoted with different letters are significantly different at p< 0.05.

Plant height was significantly higher ($p<0.05$) in the pots with wood biochar and compost together (T_6) (Fig.1). This growth improvement could be explained that compost and biochar together increase retention of water and nutrients specially nitrogen in the soil and thereby uptake of water and nutrients by the plants (Steiner *et al*., 2010). Wood biochar and compost has improved soybean plant height than saw dust biochar and compost addition treatment (T_7). Plant responses to biochar additions might dependent on the feedstock which was used in the production (Major *et al*., 2010).

Leaf numbers were significantly different ($p<0.05$) in the treatments and control, field soil without amendments (T_1) shown the least (Fig. 2). Again wood biochar addition was improved the leaf numbers (T_2 and T_6). The relative ratio of surface area to weight of the particles in biochar is thought to be one of the major determinants of its efficacy as a soil amendment, as this criterion would be reflected directly in the adsorption characteristics of biochar (Zimmerman, 2010).

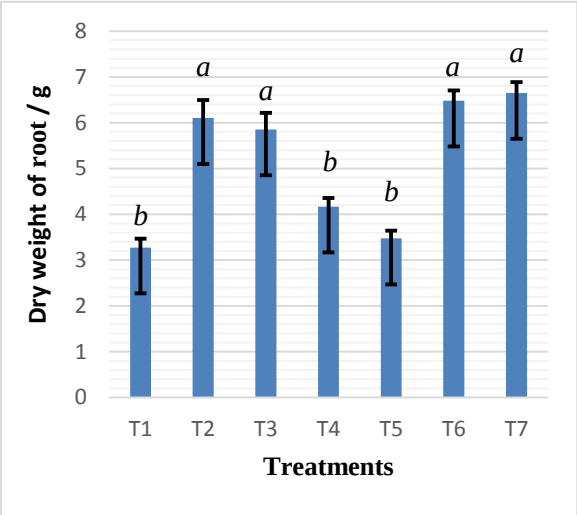

Fig.3. Root dry weight of soybean plant at different biochar, organic and fertilizer amendments. Means denoted with different letters are significantly different at p< 0.05.

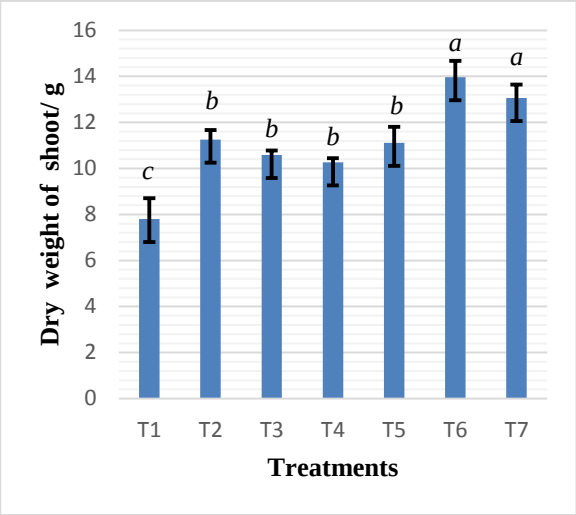

Fig.4. Shoot dry weight of soybean plant at differentbiochar, organic and fertilizeramendments. Means denoted with different letters are significantly different at p< 0.05.

Both biochar types (T_2, T_3, T_6 and T_7) were significantly increased (p< 0.05) root dry weight of soybean (Fig. 3). Similar responses of stimulating root growth with biochar were also published (Noguera*et al*., 2010). Biochar and compost together increased shoot dry weight of soybean plant significantly (p<0.05) (Fig. 4). Responses to biochar addition may be influenced shoot and root growth through a direct and interactions between biochar particles and roots. Fine roots and root hairs may take up nutrients and water from surfaces or from internal biochar pores (Lehmann and Joseph, 2009). Biochar, compost and root interactions positively influenced on structure and activity of surrounding microbial community and release or sorption of chemical signals affecting root growth (Schmidt and Noack, 2000). These biochar–root interactions could initiate a range of responses in root growth and affect shoot growth and plant performance.

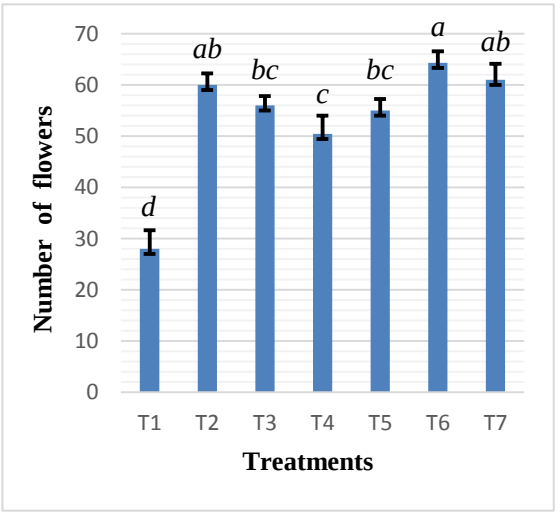

Fig. 5.Number of flowers of soybean plant at different biochar, organic and fertilizer amendments. Means denoted with different letters are significantly different at p< 0.05.

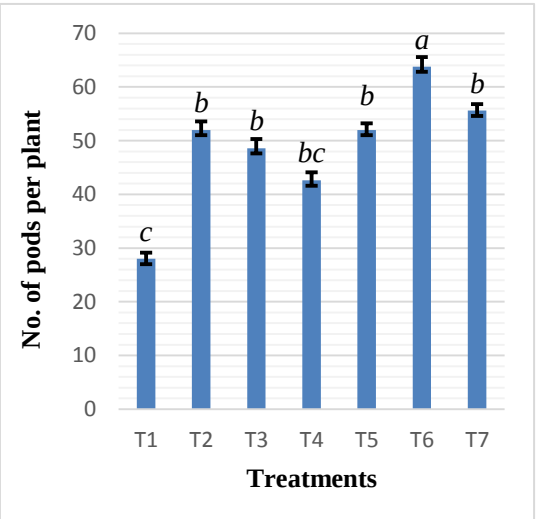

Fig.6. Number of pods of soybean plant at differentbiochar, organic and fertilizeramendments. Means denoted with different letters are significantly different at p< 0.05.

Adding biochar and compost into soybean growing soil (T_6) increased the number of flowers (Fig.5 and Fig. 6) and the number of pods of the plant significantly ($p<0.05$). It has proven that biochar and compost together synergistically improved plant yield. This result agrees with other previous studies, indicating that biochar and compost added into the soil ameliorated the growth of plant significantly and increased yield compared to non- treated soil (Sohi*et al*., 2010).

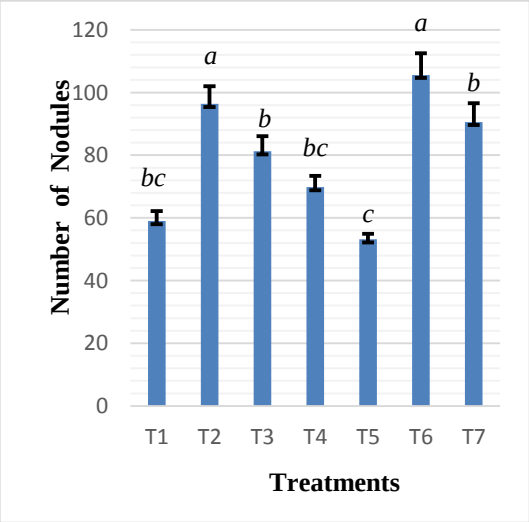

Fig. 7.Number of nodules of soybean plant at different biochar, organic and fertilizer amendments. Means denoted with different letters are significantly different at p< 0.05.

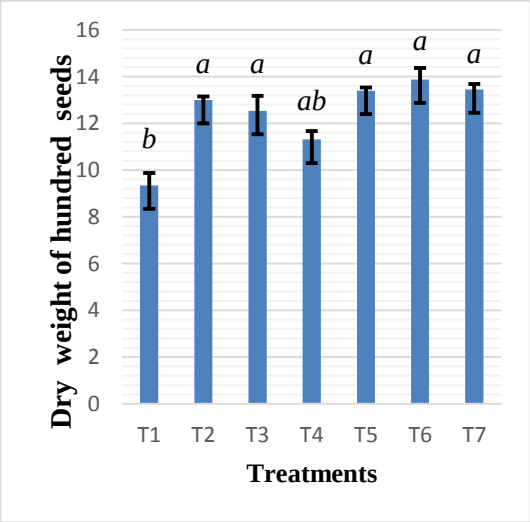

Fig. 8.Dry weight of 100 seeds of soybean plant at differentbiochar, organic and fertilizeramendments. Means denoted with different letters are significantly different at p<

Treatments of wood biochar (T_2) and wood biochar with compost (T_6) significantly increased ($p<0.05$) the nodule number of soybean (Fig.7). The treatment synthetic fertilizer (T_5) showed the least number of nodules. It has proven that availability of inorganic nitrogen negatively affect on soybean nodulation (Fig.7). Ammonium ion exchange between the biochar surface and soil solution likely to modify nitrogen availability to plant roots and may stimulate the nodulation (Liang *et al*., 2006). Dry weight of 100 seeds of soybean was significantly increased ($p<0.05$) with biochar and synthetic fertilizer treatments. (Fig.8).

Conclusion

Adding biochar into soil of soybean enhance its growth and yield and is an important alternative to chemical synthetic fertilizer. Wood biochar is promising to increase growth and yield of soybean compared to saw dust biochar. The impacts of biochar on growth and yield of soybean depend on the type of biochar. Biochar and compost together synergistically improve soybean growth and yield. Therefore, we conclude that soybean farmers can use wood biochar and compost together in the cultivation of soybean in sustainable agriculture practices.

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Review paper
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PLANT EXTRACTS AS AN ORGANIC CONTROL AGENT FOR SPIDER MITES *Tetranychus urticae* (Tetranycidae: Acarina) Koch

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Abstract

Spider mites are important pests in agricultural practices. They cannot be seen and many species are polyphag and live together. Through sucking plant juice, they can cause pale or bright dots on leaves and with intensive invasion they may cause leaf drops. Spider mites have several hosts and generally chemical pesticides are used to control *Tetranychus urticae* Koch (Tetranycidae: Acarina). However, chemicals may have severe impacts on both nature and their predators. Chemical control may also emerge pesticide resistant species. Organic control may be considered as an alternative environment-friendly tool to control *Tetranychus urticae.* Some practices like removing infested plants, proper pruning, avoiding dusty conditions, using natural insecticides and plant extracts can be considered as organic control agents.Contact impact of myrtle (*Myrtus communis* L.(Mytraceae)) extracts on *T. urticae* were tested in this study. About 3 cm diameter leaf discs from unsprayed *Phaseulus vulgaris* (L.) (beans) were used as the nutriment for the mite. Dipping method into these disks was used and experiments were conducted in 4 replications. In experiments, 2, 4, 8 and 12% doses of myrtle plants were used and counts were performed at 2^{nd}, 24^{th} and 48^{th} hours. While high rate contact was observed with 2% dose at the end of 48^{th} hour, 100% impact was observed at the highest dose.

KeyWords: *Organic control, spider mites, natural insecticides*

Introduction

Pesticides have been used against the pests for years both in the world and Turkey. These chemicals have already created serious risks on environment and human health. *Tetranchus urticae* Koch (Acarina; Tetranychidae) is a significant proliferous polyphag pest and chemicals (synthetic) are commonly used to control the pest. Negative impacts of pesticides on environmental and human health, residues left over the plants and resistance-development of the pests against the chemicals are considered as the disadvantages of pesticides. Previous researches indicated resistance development of *T. urticae* against chemicals in time (Hoyt et al. 1985; Sawickiand Denholm 1987; Keenaand Granet 1987; Herron and Rophail 1998; Van Leeuwenet al. 2004). Especially in studies carried out upto 2004, it was indicated that Tetranychidae developed resistance against 80 different acaricides (Darp, 2004).

Researchers have focused on alternative methods to overcome the negative impacts of insecticides and acaricides like resistance to these chemicals. Plant extracts and essential oils are among such alternative methods used against pests. Therefore, various plant extracts are used against pests in previous studies (Kim et al., 2003; Gökçe et al., 2007; Mahdavi Arab et al., 2008; Al-Mazra'awi and Ateyyat, 2009; Zoubiri and Baaliouamer, 2011, Çetin and Elma, 2011). Among the plant extracts, *Azadirachtin indica* A. Juss is a significant one used against pests. It was reported in previous researches that the extracts obtained from this tree were effective on more than 200 pests and insects (Kısmalı andMadanlar, 1988; Ascher, 1993; Lowery and Isman, 1993; Spollen and Isman, 1996; Bankenand Stark, 1997).

Plant essential oils containing terpenoid, alkaloid and flavonoids are also effective in pest control (Grodnitzkyand Coats, 2002; Kim et al. 2003). In a study carried out with mites,

thyme essential oil was found to be toxic for *Tetrancyhus cinnabarinus* adults and prevented egg-laying (El-Gengaihi et al. 1996).
In previous studies, together with plant extracts obtained from some weeds, essential oils of 53 different plants were also used against *T. urticae* (Tomczyk and Szymanska 1995; Kawkaand Tomczyk 2002).
In this study, leaf extract effects of *M. communis* of Mytracea family were used against Tetranychidae.

Material and Methods

The present experiments were conducted in 2015 in a climate chamber with 26 ± 1 ^{o}C temperature and 16:8 light:dark photoperiod at GOP University Agricultural Faculty. *Myrtus communis* L. (Myrtle plants) and *Tetranychus urticae* Koch individuals constituted the material of the study.
Tetranychidae individuals were obtained from stock culture over beans at GOP?? University. In experiments, 3 cm *P. vulgaris* (bean) disks were used as nutriment.
Experiments were conducted in randomized blocks design with 4 replications including a control treatment for each dose.

Extracts

Myrtle plants were supplied from Plant Protection Department of GOP University. The plants were dried at dark under room temperature and ground in a hand mill. Ground plants were supplied with methanol, shaken for 24 hours and extracted in a Soxhlet device. Then, Rotary Evaporatorwas used to evaporate methanol (Brauer and Devkota 1990).
Contact impact was investigated in extraction study and dipping method was employed. 3 cm leaf disks were dipped into extracts at different doses (2, 4, 8 and 12%) and left to dry for 30 minutes. Leaf discs were dipped into distilled water in control treatments. Dried leaf disks were then placed into petri dishes supplemented with wet cotton. Over these leaf disks, 6 mites were placed. Experiments with 4 replications were repeated 2 times. Ultimately, mites were counted at 2nd, 24th, 48thand 72nd hours.

Results and discussion

Contact impact experiments

Dipping method was used in contact impact experiments. 3 cm leaf disks were dipped into plant extracts prepared at 2%, 4% 8%and 12% doses and left to dry for 30 minutes. Leaf disks were dipped into distilled water in control treatments. Dried leaf disks were then placed into petri dishes supplemented with wet cotton. Over these leaf disks, six mites were placed on each.
Results on contact impact of *M. communis* extracts prepared in methanol are provided in Table 1.The impact rates at 2% dose were observed as 16.67% at 2nd hour, 30% at 24th and 48th hour and 53.36% at 72nd hour. The impact rates at 4% dose were observed as 26.67% at 2nd hour, 83.34% at 4th hour and 100% at 48th and 72nd hour. at8% and12% doses, 100% mortality was observed at the end of 24th hour.

Table 1.Contact impact of *Myrtus communis* L. extract on *Tetranychus urticae*

Counting time	Doses (%)			
	2	4	8	12
	Mortality Rates			
2nd Hour	16.67	26.67	26.67	40
24th Hour	30	83.34	100	100
48th Hour	30	100	100	100
72th Hour	53.56	100	100	100

Pests, mites and especially *Tetranychus urticae* are polyphag creatures causing significant damages on cultural crops, ornamental plants, recreation and forest plants and today plant extracts and essential oils are commonly used against these pests. Such extracts and essential oils usually terminate feeding, reproduction and egg-laying, they serve as a repellent and acaricide. Lee et al. (1997) reported significant fumigant impact and prevention of egg-laying of *Mentha* spp. etheric oil components. In another study, 100% mortality was reported with etheric oils including1.8-cineole, α-terpineol, verbenol and geraniol at the end of second day (Lee et al., 1997, El Gengaihiet al., 1996). Çetinand Elma (2011) investigated the contact impact of *Laurus nobilis* L plant extracts against *Callosobruchus maculatus* F. adults and assessed the impacts on prevention of egg-laying. Researchers reported LC50 value as 2.02% and LC90 value as 33.73%.

In the present study, 12% dose of *M. communis* had the greatest acaricide impact and yielded 100% mortality at the end of 24 hours.

It was concluded in this study that plants extracts and essential oils could reliably be used against spider mites as an alternative to pesticide and as a residue-free environment-friendly organic product.

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WEED CONTROL WITH ESSENTIAL OILS IN ORGANIC FARMING

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Abstract

Thymus vulgaris L. essential oil with high thymol content and *Origanum onites* L. essential oil with high carvacrol content and their equal mixture were tested for controlling of common purslane, (*Portulaca oleracea* L.), redroot pigweed, (*Amaranthus retroflexus* L.) black nightshade (*Solanum nigrum* L.) and ground cherry (*Physalis angustifolia* L.). Each essential oil was applied at the rate of 0, 40, 80, 160 and 320 L/ha in the micro-plots. The experimental design was 3 x 5 factorial with 3 replications in the micro-plots. *O.* essential oil was found more effective on the germination inhibition of redroot pigweed, black nightshade and ground cherry than those of *T. vulgaris* and equal mixture of both essential oils. However, *O. onites* and *T. vulgaris* essential oils had similar effects on the germination inhibition of common purslane. Germination inhibition of weed seeds seriously decreased with the application of essential oils higher than 80 L/ha. The results of the current study showed that oregano essential oil could be used against weeds of the cultivated areas especially in the high value organic crop growing areas**.**

Keywords: *Weed control, essential oil, germination inhibition, organic farming*

Introduction

Essential oils, composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes, are volatile natural complex secondary metabolites characterized by a strong odor (Azcan et al., 2000; Hudaib et al., 2002). Essential oils have a broad spectrum of activity against pest insects, plant pathogenic fungi, weeds and plant pathogenic bacteria. Some forms of essential oils are used as a soil drench to control weed seed germination, root pests like wireworms, while others serve as foliar pesticides applied directly to the plant itself with a range of effects from lethal toxicity to repellence.

Thymus vulgaris and *Origanum vulgare*, pleasant smelling perennial medicinal and aromatic herbaceous plants belonging to the Lamiaceae family, widely used in the Western Mediterranean countries. As a mixture of monoterpenes, major essential oil components of *T. vulgaris* are thymol, p-cymene, γ-terpinene and carvacrol (Azcan et al., 2000; Thompson et al., 2003; Giweli et al., 2013). In addition to monoterpenes, terpenoids, flavonoid aglycones, flavonoids glycosides, and phenolic acids were also found in *Thymus* spp. (Vila, 2002). *T. vulgaris* and *Origanum onites* essential oils exhibit multiple biological activities including anti-inflammatory (Braga et al., 2006), immunomodulatory (Albu, 2012) antispasmodic (Babaei et al., 2008), antioxidant (Aeschbach et al., 1994), antibacterial (Copur et al., 2010; Lakis et al., 2012), antifungal (Arslan and Dervis; 2010), antiviral (Bukovska et al. 2007), antihelminthic (Rasooli et al., 2006), carminative (Dapkevicius et al., 2002), and free radical scavenging properties (Fujisawa and Kadoma, 1992). Traditionally, the leaves of *T. vulgaris*, *Origanum vulgare* and their essential oils have been used in food industry for the flavour, aroma and preservation. Presently, an increasing interest has been expressed for *T. vulgaris* and *Origanum vulgare* because of their relatively broad biological activities. The latest researches in medicine and pharmacy have focused on developing new drugs and biomaterials based on essential oils or plant extracts, this field being a new future direction for treating

different pathologies (Bouchra et al., 2003; Baydar et al., 2004; Uremis et al., 2009; Arslan, and Dervis, 2010; Padmavathi, 2013; Haider et al., 2014).

In the organic farming systems, weeds cannot be controlled by synthetic herbicides. Hence, hand hoeing, inter-row tillage, mulching and ground covering are the major approaches to control weeds. Nevertheless, these techniques are costly and may not adequately control weeds. Consequently, naturally occurring biologically active compounds from biological origins receive more attention as a rich source of potential weed-control agents. Essential oils have a rich potential source of an alternative and environmentally safe and acceptable weed control compounds.

The purpose of the current study was to investigate the herbicdal effects of essential oils of Thymus vulgaris and *Origanum vulgare* on the germination and seedling and root growth of common purslane, redroot pigweed, black nightshade and ground cherry.

Materials and Methods

The fruits of common purslane, redroot pigweed, black nightshade and ground cherry were collected from infested areas in the experimental farm of Mustafa Kemal University in September 2013. The fruits were shade dried in the laboratory at ambient temperature (20-25 ºC) for 30 d. To break dormancy, imbibed seeds were stored for 21 d at 4 ºC in the dark.

Dry leaves of *Origanum vulgare* and *Thymus vulgaris*, grown in the Research Farm of Mustafa Kemal University Hatay-Turkey, were subjected to steam distillation for 3 h using a Clevenger type apparatus. Essential oils obtained were dried over anhydrous sodium sulphate and stored at –20ºC until gas chromatography-mass spectrometry (GC-MS) analysis.

Analysis of the essential oil was carried out by using Thermo Scientific Focus Gas Choromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 60 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 ^{o}C, MS Ionization source temperature was 220 ^{o}C, the injection port temperature was 220 ^{o}C. The samples were injected with 250 split ratio. The injection volume was 1µL. Oven temperature was programmed to 50 ^{o}C to 220 ^{o}C at 3 ^{o}C/min. The structure of each compound was identified by comparison of their mass spectrum (Wiley) using the Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of *n*-alkane standard solutions C8-C20 (Fluka, product no. 04070) and C21-C40 (Fluka, product no. 04071).

Bio-herbicide containing oregano and thyme essentials were prepared by dissolving 0, 40, 80, 160, and 320 ml oregano and thyme essential oils separately in 60 ml ethanol and 3 ml Tween 80 and sterile distilled water was added to have until 1 L volume. The commercial chemical Dasomet, (3, 5-dimethyl-1, 3, 5-thiadiazinane-2-thion), acts as fungicide, insecticide and nematicide, was used as control. Oregano and thyme essential oils were tested at 0, 40, 80, 160, and 320 l/ha concentrations, while the commercial Dasomet (Tetrahydro-3,5,dimethyl-2H-1,3,5-thiadiazine-2-thione) was used for positive control. Equal amount of oregano and thyme essential oils were mixed to have 0, 40, 80, 160, and 320 L/ha oregano + thyme essential oil. Equally mixed oregano and thyme essential oils dissolved in the sterile water as described above.

Two hundreds of common purslane, redroot pigweed, black nightshade and ground cherry seeds were mixed evenly in the sterilized soil in 0.5 x 0.5 m micro-plots. Essential oils were applied at the concentrations of 0, 20, 40, 80, 160 and 320 L/ha in the micro-plots. No essential oil was added in the negative control plots. After bio-herbicide treatment, the micro-plots were covered with polyethylene film for 7 days.

Soils of common purslane, redroot pigweed, black nightshade and ground cherry containing plots were placed in a growth chamber at 28/32 ºC for 12/12 h and dark/light period for 16/8 h. Treatments were arranged in a completely randomized design with 4 replications. After 2 weeks, number of germinated seeds were counted. Percent germination inhibition was calculated as:

$GI = [(CG-TG)/.CG] \times 100$ (1)

where, GI is the per cent germination inhibition (%); CG, germination rate in check treatment; TG, germination rate in extract treatment. Analysis of variance was performed for all data using a general linear model procedure. Data from 2 experiments were pooled and mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

All experiments were conducted in a completely randomized plot design with three replicates. The statistical analysis of experimental data was determined using a general linear model procedure of SAS for Windows (Version 8.02, SAS Institute, Cary, NC, USA), applying the one way analysis of variance (ANOVA). Mean separation was calculated according to Tukey's test ($P \leq 0.05$).

Results and Discussion

More than 28 and 27 components were identified in thyme and oregano essential oils, respectively (Table 1). However, most of the essential oil components constituted less than 1 %. Only 4 components detected in the essential oil of thyme in concentrations more than 2 % were thymol, carvacrol, caryophyllene and *p*-Cymene (Table-1). Three components in concentrations more than 2 % i.e., carvacrol, *p*-Cymene, and γ-Terpinene were detected for oregano essential oil (Table-1). The active ingredient for herbicidal activity in thyme and oregano essential oils were thymol (69.97%) and carvacrol (73.64), respectively.

Compared to negative control, germination inhibition of Redroot pigweed varied between 98.45 % and 60.30%. The highest was obtained from Dasomet and the lowest was obtained from thyme and oregano oil equal mix of 20 L/ha dose (Table 2). The inhibition rate for both thyme and oregano essential oil increased with the increased essential oil rate. When ground cherry was in consideration, the highest inhibition was obtained from Dasomet with 98.33% and the lowest was obtained from 20 L/da equal mix of both essential oil with 57.22%. Similar inhibition rates were obtained for black nightshade. The inhibition rate for *black nightshade* varied between 98.45 and 60.31%. The lowest and the highest inhibition rates were obtained from Dasomet and equal mix of both essential oil with the lowest application dose (20 L/ha), respectively. The germination inhibition of common purslane had the similar inhibition rate for both tested essential oil and their equal mix (Table 2). Inhibition rate of two essential oils on the germination of redroot pigweed, ground cherry, black nightshade and common purslane seeds were much more different at the lower concentrations, while the effects on the germination was much more less at the higher concentrations. Differences in herbicidal activity of essential oils have been attributed to differences in major active essential components (carvacrol, thymol, caryophyllene and *p*-cymene). The current study confirms the findings of Onen et al. (2002), Angelini et al. (2003), Tworkoski (2002), Kordali et al. (2008) and Uremis et al. (2009) that essential oil had great potential to inhibit seed germination and seedling growth.

Table 1. The essential oil compositions of oregano and thyme.

Components	**RT**	**RI**	**Rate (%)**	
			T.vulgaris	*O.onites*
α-Pinene	3.63	1027	0.14	0.24
α-Thujene	3.69	1031	0.18	--
Camphene	4.34	1072	0.05	0.07
β-Pinene	5.13	1111	0.07	0.23
Sabinene	5.43	1126	0.08	0.35
β-Myrcene	6.48	1169	0.36	1.98
δ-4-Carene	6.88	1183	0.41	0.08
Bornylene	7.40	1200	0.11	0.21
Eucalyptol	7.59	1208	0.28	0.8
β-Phellandrene	7.67	1211	--	2.28
γ-Terpinene	8.82	1250	2.51	**5.78**$_{\pm 0.13}$
p-Cymene	9.65	1275	**4.79**$_{\pm 0.14}$	**6.18**$_{\pm 0.19}$
α-Terpinolene	10.02	1285	0.06	0.08
1 Octen 3 ol	16.13	1453	0.21	0.17
Cis-Sabinene hydrate	16.54	1464	0.51	0.35
Camphor	18.31	1508	0.05	0.62
Trans-Sabinene hydrate	19.69	1546	0.88	0.12
Linalool	19.87	1550	0.46	0.36
Linalyl acetate	20.24	1560	0.22	0.32
Endobornyl acetate	20.85	1575	0.13	--
Caryophyllene	21.35	1588	2.69	1.05
Thymyl methyl ether	21.62	1594	--	0.9
Terpinene 4-ol	21.74	1597	1.35	1.11
Carvacrol methyl ether	22.00	1604	0.11	1.19
β-Selinene	24.02	1660	0.41	0.42
Borneol	25.33	1694	1.06	0.68
Farnesol	26.34	1722	0.32	--
Caryophyllene oxide	34.79	1966	0.88	0.25
Thymol	41.73	2216	**69.97**$_{\pm 0.25}$	**0.84**
Carvacrol	42.54	2237	**4.69**$_{\pm 0.11}$	**73.64**$_{\pm 0.27}$

Table 2. Germination inhibition of oregano and thyme essential oil on weed seeds.

Treatment	Dose	Germination inhibition, percent of the negative control			
		Redroot pigweed	Ground cherry	Black nightshade	Common purslane
Dasomet	400 kg/da	98.45	98.33	98.45	98.33
T.vulgaris	20 L/da	67.53	65.00	67.53	65.00
T.vulgaris	40 L/da	70.62	68.33	70.62	68.33
T.vulgaris	80 L/da	74.23	72.22	74.23	72.22
T.vulgaris	160 L/da	81.44	80.00	81.44	80.00
T.vulgaris	320 L/da	85.57	84.44	85.57	84.44
O.nites	20 L/da	71.13	68.89	71.13	68.89
O.nites	40 L/da	76.29	74.44	76.29	74.44
O.nites	80 L/da	81.44	80.00	81.44	80.00
O.nites	160 L/da	82.99	81.67	82.99	81.67
O.nites	320 L/da	86.08	85.00	86.08	85.00
Mix	20 L/da	60.30	57.22	60.31	57.22
Mix	40 L/da	64.95	62.22	64.95	62.22
Mix	80 L/da	75.26	73.33	75.26	73.33
Mix	160 L/da	82.99	81.67	82.99	81.67
Mix	320 L/da	89.18	88.33	89.18	88.33
LSD %5		9.80	9.82	5.56	10.57

Development of natural herbicides would help to combat with problem weeds without threating human health and environment. In this respect, thyme and oregano essential oils may be effective, biodegradable and less toxic to the environment. Based on the present results, thyme and oregano essential oils could be suggested as alternative herbicides for organic farming to control weed seedling emergence.

Conclusion

Herbicidal potential of *Thymus vulgaris* and *Origanum vulgare* essential oils were investigated on the germination inhibition of common purslane, redroot pigweed, black nightshade and ground cherry. The major active components of both essential oil had great herbicidal potential on the germination inhibition of the tested weed seeds either alone or equal mix. The germination inhibition of the tested essential oil increased with the increased concentration.

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THE PROSPECTS OF THE APPLICATION OF PREPARATIONS BASED ON BENEFICIAL MICROORGANISMS IN ORGANIC AGRICULTURE

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Abstract

In the small-plot field experiment (2012) we identified efficiency of biological preparations based on agronomically beneficial microorganisms under organic farming conditions. We studied the application of presowing treatment of sunflower’s, maize’s, buckwheat’s seeds by the biological preparation Polymyxobacterine based on phosphate-solubilizing bacteria *Paenibacillus polymyxa*, and suspension of bacteria genus *Bacillus* which has useful properties, such as nitrogen-fixing ability and plant growth-promoting properties, and also the antagonistic properties to some phytopathogenic fungi. In the soil samples taken from a plants’ rhizosphere the number of microorganisms belonging to main ecological and trophic groups, the activity of invertase, dehydrogenase, and phenol oxidase was identified. We have found out a positive effect of inoculation on the state of microbial cenosis of podzolized chernozem in the rhizosphere of plants and on the crop yields. The number of soil microflora in root zone of plants was increased by 10 – 55 %, including the increase in number of phosphate-solubilizing bacteria by 35 – 54 % or more. Enzymatic activity of soil has increased also. The extent of the positive effect of every biopreparations on the biological properties of soil and on the yield depended on the type of agricultural crops that were grown. Sunflower yields increased by 1.4 – 1.9 times, buckwheat yields increased by 1.4 – 1.6 times, maize yields increased by 1.1 – 1.2 times. In addition, in the long-term stationary field experiment (2014) we studied the efficacy of microbial preparation Polymyxobacterine at sunflower cultivation in the condition of organic farming. The number of rhizosphere microflora enhanced by 21 %, the enzyme activity increased by 10 – 20 %, the yield increased by 29 %. Our results show a prospect of application of microbial preparation Polymyxobacterine and preparation based on bacteria of genus *Bacillus* in organic farming.

Keywords: *organic farming, biological preparation Polymyxobacterine, soil microbial communities, soil enzymatic activity.*

Introduction

A shortage of high quality fertilizer based on manure of cattle and poultry in Ukraine lead to the acquiring of relevance of applicative development projects aimed at improving of soil fertility and plant yields through the application of biological preparations based on agronomically useful microorganisms that contribute to the activation of native microflora and positive soil-biological processes, optimization of plant root nutrition. The use of microbial preparations is extremely important under conditions of organic farming. These enable increase in productivity and improve the quality of crop production and provide energy and raw materials saving. A topical issue is not only the development of measures in order to improve the biological activity of the soil, but also determine the effectiveness of their application in various technologies of cultivation of crops under conditions of organic farming on different types of soils.

Our previous studies (Naydyonova et al., 2013) found that in the soil where organic farming system was used during a long term, provided only straw of different crops was used as organic fertilizers, the number of microorganisms and biological activity was reduced. In the

condition of limited resources, it is appropriate to use microbial preparations based on agronomically useful microorganisms for activation of the soil microflora.

The preparation Polymyxobacterine was created at the Institute of Agricultural Microbiology and Agroindustrial Production NAAS of Ukraine on the basis of phosphate-solubilizing bacteria *Paenibacillus polymyxa* for increasing the yield of many crops, in particular, sunflower and maize (Volkogon et al., 2006, Melnyk et al., 2007).

Miroshnichenko, Dotsenko et al. (2013) have established in a field experiments on typical chernozem that the application of presowing treatment of spring barley seeds by the biological preparation Polymyxobacterine (2004 – 2005) ensured increase of grain harvest at 18.8 %, which is equivalent to the fertilizing of phosphate fertilizers in a dose of 20 kg of active substance per hectare in the background without bacterization. Under favorable agrometeorological conditions application of Polymyxobacterine ensured of the growth harvest of green mass of corn 55 centners (5500 kg) per ha or 19.2 % compared to control. In the background without fertilizers growth of harvest of sunflower seeds as a result of application of Polymyxobacterine was 2.8 centners per ha or 12.3 % (2010).

Earlier in the small-plot field experiment (2012) we identified efficacy of various microbiological measures, including Polymyxobacterine and bacteria of the genus *Bacillus* in growing sunflowers, buckwheat and maize in organic agriculture (Naydyonova, 2014).

Bacteria of the genus *Bacillus* from collection of Soil Microbiology Laboratory of NSC ISSAR have nitrogen-fixing and plant growth-promoting properties and the antagonistic to some pathogenic fungi properties.

According to the results of the small-plot field experiment, one of the most effective measures in the cultivation of sunflower in organic farming was presowing treatment of seed by the Polymyxobacterine. But, before recommending this biological preparation for wide introduction of organic production enterprises, it needs to be tested in terms of experiments on larger areas.

The purpose of our research in 2012 was to establish the efficacy of presowing treatment of sunflower’s, maize’s, buckwheat’s seeds by microbial preparation Polymyxobacterine and suspension of bacteria of the genus *Bacillus* in their cultivation in organic farming systems. We studied the effect of preparations on the microbiological and biochemical parameters of soil, crop yield and quality of plant products and identified the most effective measures for each of the studied crops for inclusion in the technology of organic production. The purpose of research in 2014 was to identify the efficacy of Polymyxobacterine at sunflower cultivation in organic agriculture by its effect on soil microbial communities in the root zone of plants and yield.

Materials and methods

Efficiency of application of biological preparation Polymyxobacterine and bacteria of the genus *Bacillus* was studied in 2012 in the small-plot field experiment, which was laid in the long-term stationary field experiment, conducted from 1989 on podzolized chernozem in Suburb experimental field of NSC ISSAR (Korotych village Kharkiv district of Kharkiv region). Area of experimental plots was 1 m^2. Presowing treatment of seeds was performed with an aqueous solution of preparation Polymyxobacterine in compliance with the dose recommended for certain weight of every culture seeds.

Pure culture of bacteria of the genus *Bacillus* from collection of Soil Microbiology Laboratory (strain № 531) was accumulated at an appropriate nutrient medium, and then an aqueous suspension was produced and used to spray seeds of crops.

Efficiency of Polymyxobacterine in growing sunflower cultivar Jason was studied in 2014 in the long-term stationary field experiment of Agrochemistry Department of NSC ISSAR on podzolized chernozem in Suburb experimental field of NSC ISSAR (Korotych village, Kharkiv district Kharkiv region). Area of experimental plots was 168 m^2, 3-time

repetition. Organic farming system in experiment provides for the use only organic fertilizers (straw of different crops) and without herbicides means weed control.
In soil samples we identified following biological indicators: a number of microorganisms of main ecological and trophic groups by method of microbiological seeding on dense nutrient media (Zvyagintzev et al., 1980): organotrophic bacteria on the meat-peptone agar (MPA), microorganisms assimilating mineral nitrogen compounds and actinomycetes on a starch-ammonium agar (KAA), fungi on a Richter's medium, oligotrophic microorganisms on a starvation agar (SA), microorganisms, solubilizing mineral phosphates on a Muromtsev's medium, microorganisms, solubilizing organic phosphates on a Menkina's medium. Calculated indices, such as mineralization index (Mishustin, 1975), oligotrophicity index (Aristovskaya and Chudyakova, 1977) and the microbial transformation of soil organic matter (MTSOM) index (Muha, 1980), describing the tension mineralization processes and trophic regime of soil, were determined by the ratio of some groups of microorganisms, the summary biological index (SBI) by the method of relative values according to Azzi (1959).
Analyses to determine the quality of maize grains were carried out in the Instrumental Soil Research Methods Laboratory of NSC ISSAR.
Biochemical activity of soil was determined by soil enzymatic activity by photocolorimetric method: invertase activity by method set out Zvyagintzev et al. (1980), dehydrogenase by Galstyan (Haziev, 1976) and phenol oxidase according to Karyagina and Michaylovskaya (1986).
The reliability of the experimental data was assessed by means of variance analysis using the standard software package «Statistica 6.0».

Results and discussion

Results of small-plot field experiment (2012)

The use of biological preparations had a positive effect on the state of microbial communities of podzolized chernozem in the root zone of plants, both in their structure and in function. The number of most studied groups of microorganisms in soil samples from experimental plots where the preparations were used was higher than on the control plots (table 1). Thus, number of microorganisms assimilating organic nitrogen statistically significantly increased under presowing treatment of sunflower seeds by Polymyxobacterine and by suspension bacteria of the genus *Bacillus.* Also, the number of microorganisms of this group increased under the presowing treatment of maize seeds by Polymyxobacterine. Both preparations used in the experiment positively influenced on the number of microorganisms utilizing mineral nitrogen both soil under sunflower and maize. Significant effects on soil microflora under buckwheat were not found.

Oligotrophicity index which characterizing soil nutrient regime, in variants with preparations usually had lower values compared to the control that indicated the improvement of trophic regime of soil. The mineralization-immobilization of nitrogen index for soil in variants, where the biological preparations were applied, was in most cases slightly higher, that indicating a more intensive course of mineralization processes in soil. Higher values had a MTSOM index that indicated higher biochemical activity of microorganisms.

Effect of investigated preparations on the state of soil microbial communities under each crop was evaluated using SBI, which was determined taking into account number of microorganisms of main ecological and functional groups. According to values of SBI, presowing treatment of seeds by Polymyxobacterine had the greatest positive effect on the number of microorganisms in the soil root zone of maize and sunflower. Application of bacteria genus *Bacillus* had a slightly less positive effect.
Both used preparations positively effected on the number of phosphate-solubilizing bacteria, which dissolve mineral phosphates. Their number increased in comparison with control at the

beginning of the growing season by 35 – 52 % 37 – 54 % 43 – 166 % depending on the crops grown (sunflower, buckwheat, maize respectively).

Table 1 – Number of microorganisms belonging to main ecological-trophic groups in soil of plants' rhizosphere (small-plot field experiment in Suburb experimental field of NSC ISSAR, 2012)

Microbiological indicators	Crops									$LSD_{0,05}$
	sunflower			buckwheat			maize			
	1	2	3	1	2	3	1	2	3	
Organic nitrogen assimilating bacteria, million CFU/g	16.29	21.27	21.85	24.55	22.32	23.47	17.16	28.31	21.08	4.19
Mineral nitrogen assimilating micro-organisms (bacteria + actinomycetes), million CFU/g	31.75	43.12	44.11	46.11	49.37	49.89	39.39	50.00	51.75	4.63
Mineral nitrogen assimilating bacteria, million CFU/g	19.78	27.55	32.18	33.83	36.68	35.30	29.99	41.81	41.57	–
Actinomycetes, million CFU/g	11.97	15.57	11.93	12.28	12.69	14.59	9.41	8.19	10.18	2.24
Fungi, thousands CFU/g	67.87	58.42	49.85	67.30	67.67	58.82	74.41	60.81	63.18	10.59
Olygotrophs, million CFU/g	57.73	50.60	58.32	66.76	54.25	53.53	37.01	71.54	54.59	4.70
Eutrophs, million CFU/g	48.11	64.45	66.01	70.72	71.76	73.42	56.62	78.36	72.89	–
Oligotrophicity index	1.20	0.79	0.88	0.94	0.76	0.73	0.65	0.91	0.75	–
Mineralization index	1.95	2.02	2.02	1.88	2.21	2.13	2.30	1.77	2.45	–
Coefficient MTSOM	24.64	31.88	32.65	37.59	32.44	34.44	24.59	44.24	29.73	–
SBI, %	100	115	114	100	97	97	100	133	121	–

Notes to Table 1:
Numbers are average over the growing season;
1 – Control; **2** – Polymyxobacterine; **3** – *Bacillus sp*. st. 531;
CFU – colony forming units;
MTSOM – coefficient of microbial transformation of soil organic matter;
SBI – summary biological index;
LSD – least significant difference.

The important index of biological activity of soil is its enzymatic activity. Under the action of preparations activity of some enzymes increased at some extent in soil of plants rhizosphere. In the soil root zone of sunflower dehidrogenase activity increased by 21 – 27 % under the influence of application of bacterial suspension and Polymyxobacterine. In soil under buckwheat activity of this enzyme has increased by 38 – 41 % respectively. In the soil under maize dehidrogenase activity intensified under conditions of application of *Bacillus* sp. by 29 %. Invertase and phenol oxidase activity increased with using both preparations by 49 – 80 % and 12 – 28 % only in the soil under sunflower.

Increase in a number of microorganisms of basic groups in soil and increase of its biochemical activity under condition of application of microbial preparations in organic farming positively influenced harvests of agricultural plants. A harvest of dry mass of sunflower plants with treatment by Polymyxobacterine was higher than control by 71 %, anthodium by 93 %, with treatment by bacteria by 23 and 45 % respectively. Plants grown from treated seeds were higher than untreated. The harvests of buckwheat grain increased due to the treatment by Polymyxobacterine by 36 %, and due to the treatment of seed by bacteria it increased by 57 %. The harvest of raw green mass of maize increased by 4 %, raw ear maize by 16 % due to application of Polymyxobacterine, due to the treatment of seed by *Bacillus sp*. st. № 531 – by 10 and 13 % respectively.

Moreover, the use of preparations significantly improved the quality of maize grain (table 2). The most significant positive impact on the yield of sunflower and maize cobs was caused by Polymyxobacterine.

Table 2 – Quality of maize grain

Variant of experiment	Protein, %	Oil, %	Cellulose, %	Leach, %	N, %	P_2O_5, %	K_2O, %
Control	6.42	2.39	1.70	1.01	1.44	0.75	0.33
Polymyxobacterine	7.06	3.28	2.16	1.07	1.56	0.78	0.36
Bacillus sp. st. 531	7.11	2.97	2.22	1.08	1.62	0.81	0.36
$LSD_{0,05}$	0.74	0.56	0.50	0.05	0.02	–	–

Results of long-term stationary field experiment (2014)

Application of Polymyxobacterine had positive impact on microflora and biological activity of the soil in root zone of sunflower. Under action of the preparation a number of bacteria utilizing organic nitrogen was increased by 22 – 36 %, a number of microorganisms assimilating mineral nitrogen was increased by 9 – 24 % a number of oligotrophs was increased by 5 – 34 %, the total number eutrophs was increased by 12 – 27 % (table 3).

Table 3 – Impact of Polymyxobacterine on the number of microorganisms in the soil of sunflower plants' root zone under conditions of organic farming systems (long-term stationary field experiment in Suburb experimental field of NSC ISSAR, 2014)

Microbiological indicators	At the beginning of the growing season			At the end of the growing season			Average over the growing season		
	1	2	$LSD_{0,05}$	1	2	$LSD_{0,05}$	1	2	$LSD_{0,05}$
Organic nitrogen assimilating bacteria, million CFU/g	13.17	17.90	1.41	13.28	16.20	1.24	13.23	17.05	1.33
Mineral nitrogen assimilating micro-organisms (bacteria + actinomycetes), million CFU/g	38.10	47.32	4.37	47.14	51.24	4.41	42.62	49.28	4.39
Mineral nitrogen assimilating bacteria, million CFU/g	27.07	30.72	–	39.71	43.08	–	33.39	36.90	–
Actinomycetes, million CFU/g	11.03	16.60	0.62	7.43	8.16	0.58	9.23	12.38	0.60
Fungi, thousands CFU/g	49.47	63.08	8.31	28.69	23.08	5.88	39.08	43.08	7.10
Olygotrophs, million CFU/g	17.13	17.97	3.95	24.66	33.12	6.02	20.90	25.55	4.99
Eutrophs, million CFU/g	51.32	65.28	–	60.45	67.46	–	55.89	66.37	–
Oligotrophicity index	0.33	0.28	–	0.41	0.49	–	0.37	0.39	–
Mineralization index	2.89	2.64	–	3.55	3.16	–	3.22	2.90	–
Coefficient MTSOM	17.72	24.67	–	17.02	21.32	–	17.37	23.00	–
SBI, %	100	126	–	100	111	–	100	121	–

Notes to Table 3:
1 – Control; **2** – Polymyxobacterine;
CFU – colony forming units;
MTSOM – coefficient of microbial transformation of soil organic matter;
SBI – summary biological index;
LSD – least significant difference.

According to SBI values, on average the number of bacteria in the soil of root zone increased by 21 % due to application of Polymyxobacterine.

Due to increasing in the number of eutrophic microorganisms when applying Polymyxobacterine, at the beginning of the growing season oligotrophicity index decreased that indicating improvement of soil trophic regime and was essential for plant growth at the initial stages of their development. A positive aspect is the reducing of the mineralization index that causes preservation of soil organic matter reserves. Noticeable growth of coefficient MTOSM indicates intensification microbiological activity in the root zone.

Given the fact that the active bio-agent of microbial preparation is strain of phosphate-solubilizing bacteria, in the soil root zone of sunflower plants three times during the growing season number of bacteria that dissolve mineral and organic phosphates was defined. Under conditions of Polymyxobacterine application significant increase in bacteria mobilizing soil organic phosphates was observed (Fig. 1).

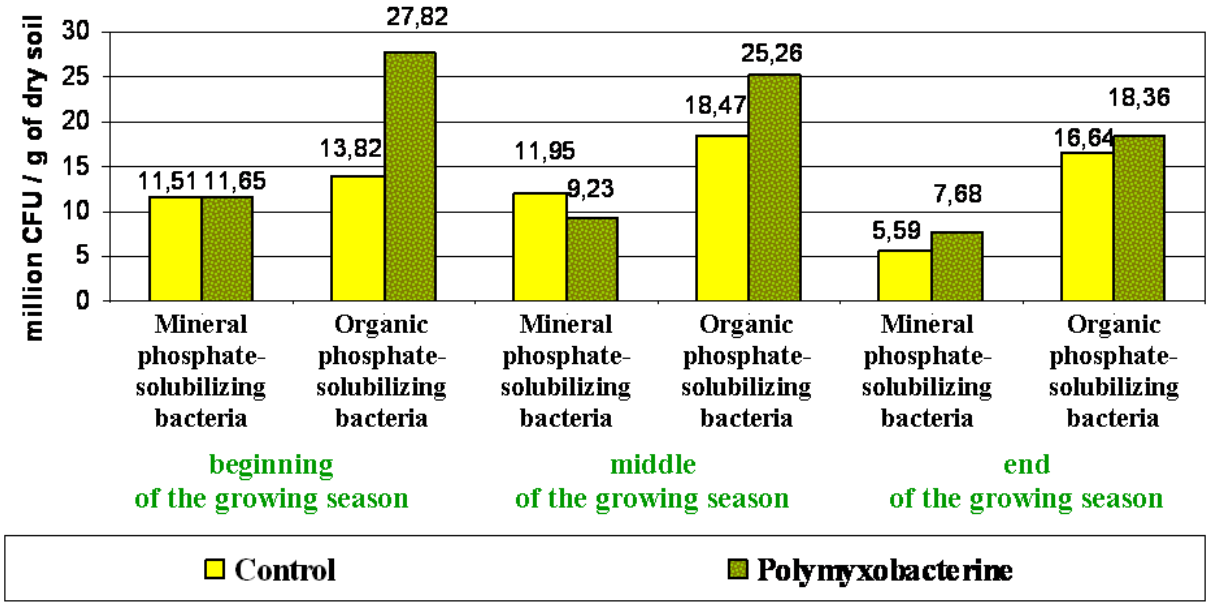

Fig. 1 – Impact of Polymyxobacterine on the number of phosphate-solubilizing bacteria in the soil root zone of sunflower plants

It is known that in addition to the direct action of microorganisms, which are part of biopreparations, their positive impact on soil root zone of plants is to enhance native soil microflora, resulting in increase of its biochemical activity (Volkogon et al., 2006).

Positive effect of Polymyxobacterine on the enzymatic activity of the soil in the root zone of plants was noted (table 4).

Invertase activity was increased at the beginning of the growing season by 11.6 %, at the end of the growing season it increased by 83.6 %. The most noticeable phenol oxidase activity was increased due to the use of Polymyxobacterine. It was greater than control in all periods of observations: at the beginning of the growing season by 10 %, in the middle of the growing season by 15.7 %, at the end of the growing season by 19.6 %. Increase in phenol oxidase activity is particularly important for organic farming, given that phenol oxidase is involved in the synthesis of humus components, so increase in its activity determines the orientation of biochemical processes in the direction of preservation and stockpiling of its reserves in the soil.

Table 4 – Effect of Polymyxobacterine on enzymatic activity of soil root zone of sunflower plants (2014)

Variant of experiment	Dehydrogenase, mg TPF / 100 g per 24 hours			Invertase, mg glucose / 1 g per 24 hours			Phenol oxidase, mg 1,4-n-benzoquinone / 10 g per 1 hours		
	1	2	3	1	2	3	1	2	3
Control	157.50	41.83	133.50	2.41	9.45	2.14	600.00	547.23	612.00
Polymyxobacterine	145.50	46.27	127.50	2.69	9.00	3.93	660.00	633.16	732.00
$LSD_{0,05}$	14.86	4.02	9.68	0.19	0.52	0.34	29.20	26.14	34.97

Notes to Table 4:
1 – at the beginning of the growing season; **2** – in the middle of the growing season; **3** – at the end of the growing season;
TPF – triphenylformazan;
LSD – least significant difference.

Activation of native microflora and positive soil-biological processes was effected on the yield of sunflower (table 5). Application of Polymyxobacterine ensured sunflower yield increase by 29 %.

Table 5 – Effect Polymyxobacterine on the yield of sunflower, centner · ha^{-1} (2014)

Without biological preparation	Application of Polymyxobacterine	LSD $_{0,05}$	Increase of yield	
			centner · ha^{-1}	%
32.38	41.67	4.82	9.29	28.69

Conclusion

In small-plot field experiment (2012) positive impact of seeds inoculation by Polymyxobacterine and by bacteria of the genus *Bacillus* on the number of soil microflora and soil enzymatic activity in root zone of sunflower, buckwheat, maize plants was established. The degree of positive influence of each type of treatment on the biological properties of soil and crop yield depended on the grown crop. Strain № 531 of bacteria genus *Bacillus* from collection of Soil Microbiology Laboratory of NSC ISSAR has potential as bio-agent for the developing of a biological preparation based on it.

According to the results obtained in the long-term stationary field experiment (2014), a significant positive effect of biological preparation Polymyxobacterine for the number of microorganisms in the root zone of sunflower plants under organic farming condition was identified. On average, number of investigated microorganisms increased by 21 %. Also soil enzymatic activities significantly increased: invertase by 12 – 84 %, dehidrogenase by 11 % phenol oxidase by 10 – 20 %. Sunflower yield was increased with use of Polymyxobacterine for 9.29 centner · ha^{-1}. The number of agronomically valuable phosphate-solubilizing bacteria significantly increased too.

The economic effect of the use Polymyxobacterine in sunflower growing under conditions of organic farming is in increasing harvest by 28.69 %. However, environmental effect is also valuable for the organic farming that means environmental cleanliness maintenance and preventing soil pollution.

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STUDY OF SOME BIOLOGICAL MICROBIOLOGICAL CHARACTERISTICS OF SOIL AND RHIZOSPHERE FABA BEAN (Vicia faba L. vr equina and minor)

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Abstract

Studies on microorganisms have in recent years to develop the concept of "organic fertilizers" based primarily on properties known as "beneficial" in some organizations. Apart from these nitrogen-fixing bacteria widely used for making organic fertilizers are capable of some dissolved minerals in rhizosphère. These bacteria find a land application in agriculture in structuring the soil rhizosphere species *Vicia faba* beans or kidney beans (v v equina and the minor), presents an excellent host rotation enriches the soil with nitrogen by root excretion of nitrogenous products by detachments nodule or remaining roots.

Before culturing the eleven floors of this rough textures, and heavy, after chemical and physical fronts v f crop Vicia faba equina and minor crops were put in the two species separately and has made the same physico -chemical, it was noted that the rate of carbon and nitrogen falling on almost all soils after cultivation.

The resulting C / N ratios vary, but the rate of phosphorus are significant and increase in both types of cultures nodulation tests were positive with a slight difference noted in favor of the minor variety, it rots due to be rhizoidal diversity of flora which is at the origin of nodulation and that would be specific to this variety has rather to the overall charge of this flora latter finding at least partly explain the difference in nitrogen levels between the two types cultures, which could be related to deficiencies flora associated symbiotic.

the decrease was less pronounced in the variety minor compared to the variety equina by against the latter seems more preferment viewpoint nitrogen fixation.

Keywords: rhizobia, symbiosis, nodule, fixing

Introduction

Soil is a real habitat, natural environment where plant roots dominate in bacterial and fungal microflora (Raoul, 2003). In soil and root soil interface, trace elements undergo various transformations related to their property and under the control of physical, chemical and biological parameters and often influenced or controlled by the action of microbial soil community and more particularly in the rhizosphere of plants (Deneux- Muslin et al, 2003). Among the species found in legumes Vicia faba or fava beans (V. equina L. and V. minor L.) that perfectly illustrates the symbiotic interaction with rhizobia agronomically. In view of the paucity of studies targeting two varieties mentioned, this study is a contribution to the knowledge of existing biological and soil interactions before and after culture of faba beans. Legumes have an important plant family in the world since agriculture include all protein and are involved in the improvement and enrichment of soil nitrogen (Nour, 1995).

Materials and methods

1 Soil Analysis

1.1. Soil collection N Sampling sites

Soils used in this study were obtained from eleven (11) locations in the *Province of Bejaia (Algeria) (Fig..1) *

Fig.1: Location map of soil sampling sites

1.2. Preparation of fine soil

The amounts collected are dried in the open air before undergoing manual crumbling.

1.3. The culture of faba

I carried out the seedlings Both varieties of faba bean (equina and minor). Culture lasted two months during which we kept watering and regular monitoring.

1.4.Physical analyzes

1.4.1. Grit

This is to determine the statistical distribution in a sample of fine soil (Φ <2mm) size fractions of five data according to diameter classes according to the scale of Atteberg defining classes following different diameters (Baize, 1997; Mc Sweeney, 1999)::

1.4.2. pH measurement

The pH is measured by electrometer (pH monitoring) on a soil solution which pondéro-volume report soil / water is 1 / 2.5.

1.5. Chemical analysis

1.5.1. Determination of Organic Carbon

The organic carbon content is determined by the Walkley-Black method; further modified method called Anne (Dugain et al, 1961;. Baize et al, 1995.) ..

Then, an excess dichromate ion is titrated with a solution of ferrous salts to 8% (Mohr salt). The rate of OM is derived by multiplying the percentage of organic carbon by u

n factor of 1.72 (MO = 1.72 * ROD).

1.5.2. Determination of Total Nitrogen

The assay is performed on a sample of 1 g of fine earth by the Kjeldahl method, the protocol is as follows (Kaouritchev, 1980):

- Mineralization (Norrg ammoniacal N2) in the presence of hot concentrated H2SO4, and a mixture of catalysts (Se, K2 SO4 and CuSO4).
- Distillation in the presence of NaOH (40%) and H3BO4 (4%).

Titration with a cold solution of H2SO4 (N / 50). (Appendix 5).

1.6. Expression of Results:

All results except pH, are usually expressed per 100 g of fine earth: Different size fractions (A, LF, LG, SG and SF) and organic carbon (C ANNE), comparable in% = g / 100g TF

- Total nitrogen (NKJELD.) = ‰ in g / kg of m.p.

1.7. Bioassays:

a-Counting nodules

having dug up the plants in each pot until the total removal of the soil. We counted the number of nodules in both varieties equina (E) and minor (M) grown in 11 soils.

The location and shape of nodules on the root system are photographed to be described hereafter

Results

2- Results of physical and chemical analysis of soil

floors	Clay (%)	Fine silt (%)	Coarse silt (%)	**fine sands (%)**	**T.1: Table of different soil textures coarse sands (%)**	**Texture**
AKB	25,32	27,98	4,76	11,55	30,39	*SLA*
AMT	31,81	37,11	7,74	8,58	14,74	*L A*
AOK	36,88	15,8	3,37	4,74	39,19	*A S*
BER	46,63	25,9	11,19	7,46	8,8	*A*

CHE	30,48	20,32	5,89	27,13	16,15	*SAL*
ELK	21,17	43,43	4,45	15,74	14,65	*L A*
FER	41,32	10,33	7,54	18,59	22,21	*A S*
KHE	54,4	24,18	5,56	9,79	6,04	*A*
SEM	51,13	28,4	6,81	7,5	6,13	*A*
TIC	36,19	15,51	6,61	19,64	22,02	*A L*
UNI	33,22	22,14	7,86	18,71	18,05	*A L*

2.3. Organic carbon

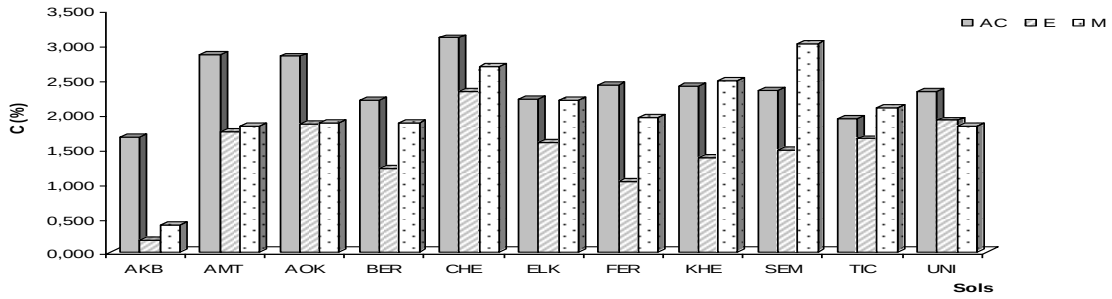

Fig.2: Variation percentages of organic carbon in different soil

2.4. Total nitrogen

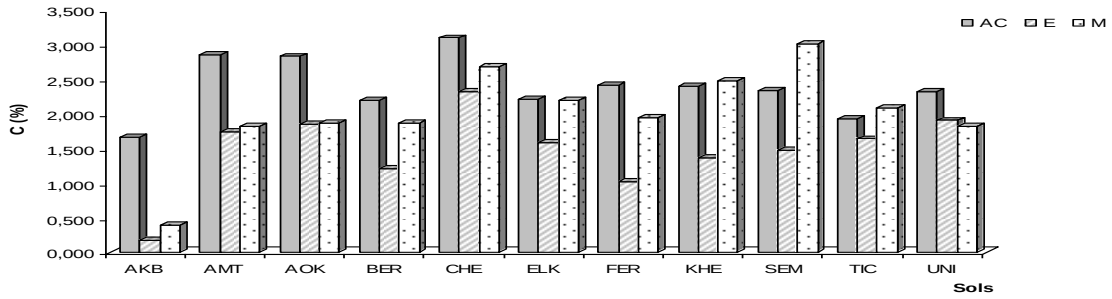

Fig. 3: Variation percentages of organic carbon in different soil

3. Bioassay results

3.1. Shape and location of nodules

Racines – *minor*

Racines – *equina*

Nodules – *minor*

Nodules – *equina*

3-2. Observation and result

Our watches these images that there is a variety diferences between both minor and equina roots of minor and more than nodulate therefore equina symbiosis and very strong in minor than equina

3.3. Plants soil microorganisms Interactions:

/	N	C	C/N	P	number nodules
Variety equina	+	-	variable	++	++
variety minor	++	+	variable	++	+++
before Culture	+	++	+	-	/

T4 . Variations of the main parameters depending on the cultivar

Conclusion

These results offer us two perspectives, first from a purely agronomic by enriching the soil with nitrogen and the other on that of basic research and should note any time that these variations do not follow the same rate on all the parameters covered in this study; it appears that the soil is a complex and dynamic environment, integrating all living beings in its own operations and in all interactions with wildlife

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DIRECT SEEDING EMERGENCE IN THE AGRICULTURAL FARMS OF THE SETIF PLAIN (ALGERIA)

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Abstract

The conservation agriculture concept enables to define different production systems. Its selection and application depends on soil properties, climate and socio-economic considerations. Direct seeding was firstly introduced in Algeria to improve the wheat production in the high plains of Setif and by the “Large Crops Technical Institute” which is a state agency provided assistance and technical support to farmers in this purpose.

Surveys were conducted through a representative set of ten cereal farms in the Setif agricultural plains during the 2014/2015 season. The objective was to describe the agricultural practices associated to the direct seeding and analyze their possible effects on the environment.

Our first results show that the agronomic yield has been improved compared to the traditional technique of seeding in addition to some improvements in organic matter, water retention, biological activity and especially limiting erosion. However, we observed that the use of chemical herbicides (glyphosate) has adverse effects on soils, crops and even humans.

Keywords: *Conservation agriculture, direct seeding, efficiency, glyphosate, Setif.*

Introduction

The cereal sector is one of the main sectors of agricultural production in Algeria. The Setif plains were considered since a long time as the leading for cereal production in Algeria. They have large areas of agricultural lands and receiving amounts of rainfall enabling wheat production. However, yields are generally low due to declining soil’s fertility. The main factors that predisposed to this degradation are: water deficit, overexploitation of agricultural lands and actual farmer’s practices. To increase the production, the “Large Crops Technical Institute” of Setif (L.C.T.I) which is the Algerian pioneer development agricultural institution introduced in 2002 direct seeding to spread the conservation agriculture in public and private agricultural farms. In this framework, several studies were conducted particularly the characterization of some parameters of soil fertility and crops behavior (Ziza, 2007). The author described the direct seeding introduction and diffusion in the Setif plains and presented the expected are the effects of this new agricultural technique. To carry out our work, we implemented a comprehensive approach based on farmer’s survey and physical measurements.

Material and methods

The Setif agricultural plains are located north eastern of Algeria (figure 1). The climate is continental and semi arid with cold rainy winters and hot summers and rainfall is the unique irrigation resource. This context is responsible of high evaporation, soil erosion and soils fertility decrease. Field surveys were conducted over the study area of 1011 ha during the agricultural season 2014/2015.

Our investigations focused on 10 agricultural farms covering 381 ha which are practicing with direct seeding and one aim of this study was to assess the possible impacts of these practices on soil properties. Semi open questions were asked face to face to a sample of 10 farmers involved with durum wheat cultivation by direct seeding.

The agricultural farms were selected to include all the sub areas of the study site according to precipitation magnitude: northern farms receiving an average rainfall varying between 500 and 400 mm, high plain farms receiving an average rainfall between 400 and 300 mm, southern farms which were subdivided into two groups, farms receiving between 300 and 200 mm and farms receiving less than 200 mm.
According to Lahmar et al. (1993) in Karkour (2012), “the whole soils of the studied region are carbonated. The northern part is covered by calcareous soils whereas in the region of high plains soils are calcic type, rich in clay and poor in humus in their northern part and turn into stony in their southern part”. Farmers were asked about their actual and past practices, their farm’s characteristics like soils properties before the emergence of direct seeding. The main observations about the introduction of direct seeding and the dominant weeds and treatment used were recorded and finally others problems dealing with durum wheat development in the region. In consistency with the literature review, organic matter lost, biological activity and water retention level were assessed.

Figure 1: Location of Setif plains and the study area

Results and discussion

During the survey, we noticed that 60% of the farmers have secondary level of education and 40% have a high level of education. This factor may probably impact the farmer’s adoption capacity of direct seeding. They generally came to the agriculture by heritage (family links) and they seem enthusiastic to use direct seeding. When the question “why did you introduce direct seeding?” many answers were provided among which the following are listed:
- "The direct seeding which was introduced in Large Crops Technical Institute of Setif in the framework of a collaboration with the International Center for Agricultural Research in the Dry Areas gives good results. This institution is strongly involved in developing durum wheat production in the Middle East and North Africa. Its local staff provided a technical support in relation with this technique".
- "The direct seeding is not perceived as a new technique. It is an amelioration of the old technique, because before apparition of the plough our grandfathers had used plow to cut soil and make seeds, after that they lock soil by their foot, so they had not till soil deeply".
The other information kept from field is related to the herbicide applied to the soils when seeding. Most of farmers use roundup and/or glyphose depending on its availability in the national market. The active matter is glyphosate with chemical formula is C3H8NO5P with a concentration of 360 g/l. The herbicides are used with different amounts from a farmer to another.The determination of an amount depends of the rate of weeds before seeding and the farmer's purchasing power.
The pioneer farmers started with an amount of 6l/ha and currently all farmers apply an amount that ranges from 3to 1.5 l/ ha. The concentration of the active matter due to the herbicide which depends on the amount applied and the duration of the application was

calculated (table 1). According to the results, there are 03 groups of agricultural farms: low active matter group (< 1 kg/ha), medium group (between 1 and 6 kg/ha) and high active matter group (> 6 kg/ha). This result should affect the agronomic performance in terms of yield and the environmental one in terms of impacts on soils sustainability.

60% of the surveyed farmers who introduced direct seeding in 2005 found that direct seeding increase the content of organic matter in the soil in agreement with the conclusion of Belagrouz (2013). Boudiar (2013) calculated the total carbon in similar conditions of direct seeding use and found an amount of 39.60%. Zetal (2011) assessed the cumulative of organic matter in a sub humid climate due to direct seeding and found it ranging from1.99% to 1.60% respectively from the surface horizon to the deep. The surveyed farmers have unanimously listed the water deficit -and consequently its conservation in the soil- as the top issue for a good production. In fact, Mrabet (2001) stated that the direct seeding improves the storage of water of rainfall from 10% to 30% when compared with conventional technique. The Water retention capacity of the soil is linked to its structure and rate of organic matter. This point has been studied in many researches and confirms the amelioration of retention capacity of water with direct seeding in the region of Setif. Moreover, 70% of the surveyed farmers noticed an amelioration of soil especially in dry season. In 2011, Zetal (2011) has evaluated water retention capacity with a comparative study between direct seeding and conventional technique, the results are that "The direct seeding preserves water in the soil compared with conventional technique with a gap to 3%, during the development cycle of wheat, humidity varied between 22% and 15% and water retention capacity is very significant in direct seeding, it varied respectively from 9.8%-20.7% and 8.6% respectively from the surface horizon until deep horizon".

The second indicator of soil fertility is the biological activity. A few results about the development of a biological activity in the region of Setif are available particularly in the case of the direct seeding (Ziza, 2007). However, farmers claim that some soils lombiriciens are regularly observed.

More over farms located between isohyets of 300 and 200 mm i.e the dry part of the study area, suffer from soil erosion. Farmers state that direct seeding has limited this phenomenon. They confirmed that direct seeding was mainly introduced in Setif in 2002 to limit erosion. Belagrouz (2011) said that "the direct seeding system is a promising technique to protect the soil against erosion”. Another question has been asked regarding the weeding operation in post seeding. "What do you think about the weeding operation in post seedling and the herbicide than you have used?" According to the farmers, the treatment of weeds by glyphosate before seeding preserves the land during the whole of the cycle development of wheat and reduces costs of weeding. However, this may be responsible of no germination of weeds before seeding particularly when rainfall is irregular. In these conditions, the farmers apply the herbicide just after seeding, but before apparition of the wheat seeds.

Table 1: Active matter of glyphosate in the study farms of Setif 2014

Glyphosate application duration (in years)	Amount of glyphosate per area	Concentration of active matter (kg/ha)
1	10,5 kg/12 ha	0,875
2	17.15 kg/25 ha	0,660
7	73.5 kg/10 ha	7,350
8	728 kg/130 ha	5,600
8	369,6 kg/66 ha	5,600
8	16.8 kg/3 ha	5,600
9	1176 kg/120 ha	9,800

Conclusion

The present research enabled to explore the context of the emergence and diffusion of the direct seeding in Algeria. Agricultural farms were classified in three groups according to the amount of herbicide, agronomic yield and soils properties.

Field observations shows that direct seeding is a good solution for some parameters of the soil in particular erosion, water retention, organic matter and biological activity.

Although, natural and human constraints listed by farmers surveyed like the lack of water resources, the lack of permanent vegetation in soil and the poor weed management plays a negative role for the direct seeding management.

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ANTAGONISTIC ACTIVITY OF TWO STRAINS OF BACILLUS SP. OBTAINED FROM AN ALGERIAN SOIL AGAINST THE MIGRATORY LOCUST LOCUSTA MIGRATORIA (LINNAEUS 1758)

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Abstract

The aim of our study is to isolate, purify and characterize two entomopathogenic bacterial strains of the genus Bacillus from the soil of Adrar in the Algerian Sahara. The molecular characterization of the isolated strains was carried out by DNA isolation, PCR and sequencing of 16S rRNA gene, followed by a phylogenetic analysis. After that, the study of the bacteria toxicity on the fifth larvae stage of Locusta migratoria was conducted by assessing rates of mortality, LT_{50} and $LC_{50.}$ The phylogenetic analysis showed that the two strains (Strain-B1 and Strain -B2) were identified as Bacillus sp. (HE799656) and Bacillus sp. (HE805963), respectively, with a very high similarity (99%) with the strains Bacillus thuringiensis (NR_043403) and Bacillus weihenstephanensis (NR_024697). Besides, the results of toxicity tests showed that larvae of L. migratoria are influenced by the two isolated bacterial strains. This action is more pronounced with the Bacillus sp. strain-B1 (HE799656) compared to Bacillus sp. strain-B2 (HE805963). Examination of TL_{50} shows that these times vary depending on the bacteria and concentrations applied. Similarly, the LC_{50} are closely related with time and they also vary depending on the bacterial strain tested.

Keywords: *Locusta migratoria, Bacillus sp*., larva-mortality.

Introduction

Among the locusts' enemies of Sahelian cultures, the migratory locust *Locusta migratoria* (Linnaeus, 1758) (*Orthoptera, Acrididae*) is the major pest invasion period. The damage is mainly limited to grass, increasing the risk of social erosion and poverty (Zakaria *et al.,* 2003). The migratory locust occupies a very large geographical extension. Many subspecies have been described mainly in Africa, Madagascar, Oriental Asia, Australia and Mediterranean regions (Duranton *et al.,* 1982). Locust remains a major concern in the strategies of crop protection (Zakaria *et al.,* 2003). However, current methods of control using insecticides are very effective in the desert but also harmful to many other animal species biotope and the environment. In this context, the use of insect microorganisms with insecticidal effects offers some potential (Joung & Cote , 2000).

It is in this concept that this work falls. It aims to isolate bacterial strains belonging to *Bacillaceae* group, of the same biotope as the locust's, to assess their biological impacts on the later by determining the rate of mortality and calculating LC_{50} and LT_{50}.

Material and methods

Soil sampling

Sampling was achieved from soil cultivated in the region of Adrar in the Algerian Sahara soil in September 2013. Samples were taken at the rhizosphere layer.

Isolation, identification and characterization of bacterial strains

Different soil samples are separately dried, screened and finely ground. Of each sample was weighed 10 g to suspend in 90 ml of sterile physiological saline water, whilst stirring. Thereafter every suspension undergoes a series of decimal dilutions (10-1 to 10-5) (Guiraud, 2003). The purity is checked after each passage by microscopic examination of a smear stained with blue methylene and having undergone a Gram. The identification of purified isolates has been subject of a multiphase study, comprising a macroscopic, macroscopic, physiological, biochemical and molecular study of characters.

Molecular identification

DNA extraction, PCR and sequencing of 16S RNA

Extraction of DNA was performed as described by Ausubel *et al*. (1988). The extracted DNA is electrophoresed on agarose gel at 1% for 30 minutes under an electric current of 100V. The DNA was subsequently recovered from the agarose gel and purified using the Jetsorb extraction gel kit (Genomic DNA purification system-PROM, EGA).
The purified DNA is amplified by a Hot Start PCR (94 ° C) using specific primers eubacterial 16S rDNA primers (forward primer 5'AGAGTTTGATCCTGGCTCAG3' (*Escherichia coli* positions 8-27) and reverse primer 5'ACGGCTACCTTGTTACGACTT3' (*E. coli* positions 1494-1513) (Weisburg *et al* 1991). Each 50 µl reaction volume is composed of 2µl of PCR buffer, 0.5 mM of each primer, 1.5 mM MgCl2, 50 mM of each deoxynucleoside triphosphate and 1 µl of Taq polymerase.
The samples analyzed are deposited into the wells of 50 µl PCR plates, installed in a thermocycler type Mastercycler Personal (Eppendorf, Germany). The following program was used: 95 °C for 1 min, followed by 40 cycles of denaturation (1 min at 95 °C), annealing (1 min at 55 °C) and polymerization (2 minutes at 72 °C) for 5 min.
The amplification products are purified using the PCR DNA GFXTM Kit and Gel B and Purification Kit (Amersham Biosciences) then cloned into a vector based on the instructions of manufacturer's instruction (INST / AcloneTM PCR Product Cloning Kit, MBI Fermentas). Transformed clones were selected on LB medium containing ampicillin (100 mcg / ml) and X-gal (80 mcg / ml).

Phylogenetic analysis

The 16S rRNA gene sequences obtained after sequencing were subjected to a similarity search of sequences deposited in the Genbank database (on the NCBI National Center for Biotechnology Information website) using the BLAST program. The multiple sequence alignment was performed by CLUSTALW 1.8 (Thompson et *al*., 1994). The sequence homology was then evaluated and the phylogenetic tree was created by neighbor joining method (Saitou & Nei, 1987) using the MEGA 5 software (Tamura *et al*., 2011). The confidence levels of the topology of the phylogenetic tree obtained were estimated by the analysis of data resampling methods (bootstrap) with 1000 replications.

Evaluation of the toxicity of the bacteria on larvae L5 of *Locusta migratoria*

Source of locusts

The study is performed on L5 larva of the species *Locusta migratoria*. These are captured in the desert of Algeria and put into mass breeding in the laboratory.

Preparation of bacterial suspensions and application of biological treatments

To highlight their entomopathogenic power towards the migratory locust, we have initially conducted the preparation of the mother-solutions. Successive dilutions are then prepared to

10^{-4} dilution. For the application of biological tests, 48 hours old larvae are treated after fasting for 24 hours orally (Oulebsir-MohandKaci, 2012).

Calculation of mortality percentages

The percentage of mortality observed in the control and treated larvae is calculated using the following formula:

Observed mortality = (Number of dead individuals / Total number of individuals) X 100

Calculation of LC_{50} and LT_{50}

Before calculating the TL_{50}, the percentage of observed mortality is corrected relative to the control in the form of ABBOT (1925).

To calculate the LC_{50} (concentration needed to kill half of a population) for each bacterium, we have transformed the concentrations used in logarithms and mortality percentages for corrected probits by using the table probit. The LC_{50} is determined from the equation of the regression line.

Statistical analysis

To confirm the effectiveness of the biological treatment performed, the results are subject to the test analysis of variance. We have applied the Tuckey. The software used is the XLSTAT.

Results and discussion

Physiological and biochemical characterization of bacterial isolates

The multiphase study, being initiated by exploring the macroscopic appearance of bacterial cultures grown on nutrient agar, has shown that isolates carrying the B1 and B2 codes provide well-isolated colonies, broad, smooth, flat, circulars, cream color with irregular edges. Observation under the light microscope of fresh cells and after simple staining with methylene blue and Gram stain, has shown that the two strains are Gram positive and are long stick shaped with square end. Alongside the microscopic study, malachite green staining revealed that both isolates have a spore form. The spore has an oval shape nondeforming center position. Moreover, isolates B1 and B2 have shown positive results for the test of catalase, nitrate reductase and mannitol-mobility. The results of the pyruvic acid derivatives appeared positive for the Voges Proskauer reaction and the two isolates show a positive response for the hydrolysis of starch, gelatin and casein. In contrast, strains B1 and B2 expressed negative hydrolysis test against the indole and the Simmons citrate, and strain-B2 had not hydrolyzed urea. Both strains showed growth after culture and incubation at 45 °C, contrary to the incubation at 55°C and 65 °C which showed negative results.

Isolates have all the cultural characteristics of the genus *Bacillus* already described by Brossard & Terry (1984) and Euzeby (2007). The specific classification has helped join the two bacterial strains isolated from the ground of the Algerian Sahara to the genus *Bacillus* (Guiraud, 2003). Partial sequencing confirms this affiliation with a very high similarity to *Bacillus thuringiensis* (NR_0434030). Indeed, the majority of bacteria of the genus *Bacillus* live in the soil or they persist with their spores. These are bacteria from land who derive their nutritional needs from an organic matter, nitrogen and minerals present in the soil (Brossard & Terry, 1984; Ashnaei *et al*., 2009).

Phylogenetic analysis

The phylogenetic position of the two strains (Strain and Strain-B1-B2) is represented in a phylogenetic tree drawn by the neighbor joining method (Figure 1). The bar represents one substitution per 100 nucleotides. Level nodes of the values indicate the probabilities calculated by bootstrap. The 16S rDNA sequences of the two strains were obtained with 760 and 1504 nucleotides and stored in the database EMBL / EBI under reference numbers HE799656 accessions and HE805963 for the strain B1 and B2, respectively. Sequence analysis of these two strains has shown they are related to organisms belonging to the family

Bacillaceae. They are affiliated to the genus *Bacillus* showing very high sequence identity (99%) with the strains *Bacillus thuringiensis* (NR_043403), *Bacillus cereus* AM7 (JQ435684), *Bacillus weihenstephanensis* (NR_024697.1) and *Bacillus mycoides* (NR_036880). The two strains are in the same cluster as that of *Bacillus thuringiensis* (NR_043403) and *Bacillus cereus* AM7 (JQ435684).

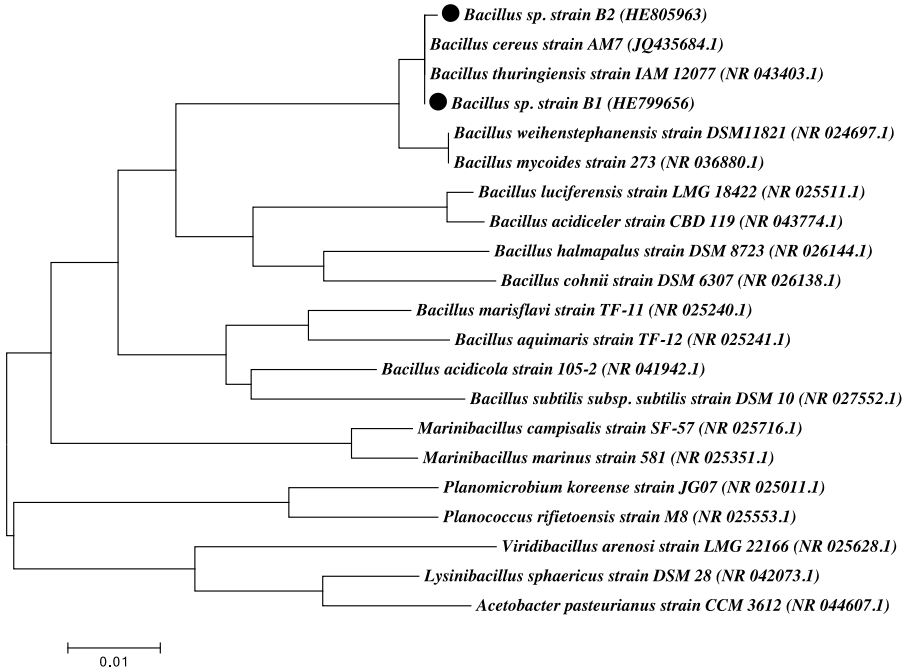

Figure 1: Phylogenetic tree drawn by Neighbor-Joining method of showing the phylogenetic position of strain-B1, strain-B2 and representatives of some other related taxa based on 16S rDNA sequences.

Evaluation of the toxicity of the bacteria on the larvae of *L. migratoria*

Effect on Mortality

Within L5 larvae treated with *Bacillus sp*. strain-B1 (HE799656) (Figure 2), mortality reached 100% after 12 days after treatment at the high dose D1 and at 21 days after treatment at the intermediate dose D2 and finally 90% mortality obtained the same day after treatment with low doses D3. Similarly, mortality rates ranging from 86.67% after treatment with high dose to 70% after treatment medium and low dose of *Bacillus sp*. strain-B2 (HE805963) are achieved after 22 days (Figure 3). Analysis of variance revealed a very highly significant difference at 5% level ($P < 0.0001$) between the control batch and the treated batch by both tested bacteria. Similarly, the Tukey test revealed significant differences for all combinations.

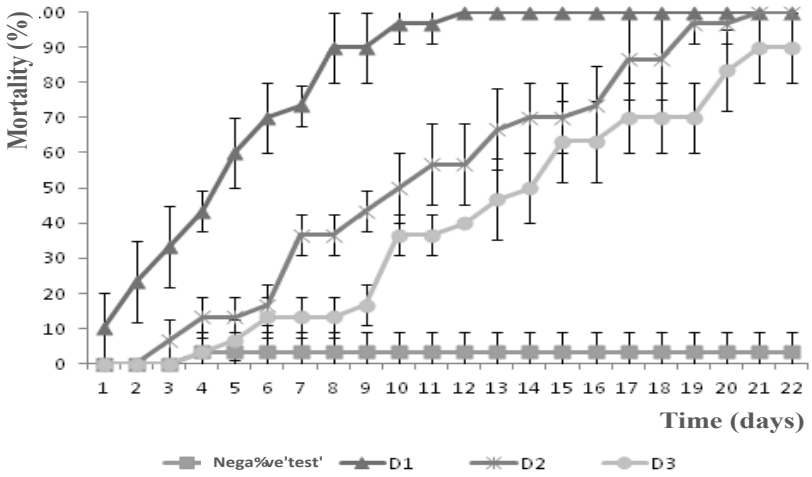

Figure 2: Cumulative daily mortality rate L5 larvae of *L. migratoria* treated with *Bacillus sp.* strain-B1 (HE799656) at doses D1 =1.6 mg/ml, D2 = 0.59mg/ml, D3=0.33 mg/ml.

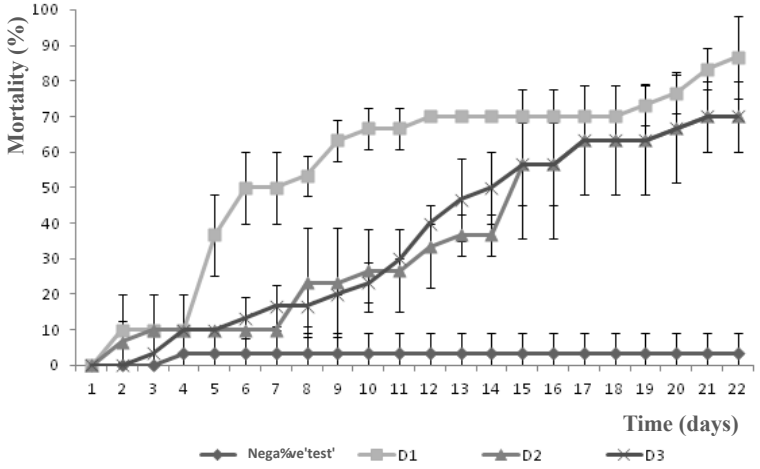

Figure 3: Cumulative daily mortality rate of L5 larvae of *L. migratoria*t reated with *Bacillus sp.* strain-B2 (HE805963) at doses D1= 1,67 mg/ml, D2=0,62 mg/ml, D3=0,35 mg/ml.

In the light of results, and for this parameter, it appears that larvae are influenced by our two bacterial strains. This action is faster with the strain *Bacillus sp*. strain-B1 (HE799656) compared with *Bacillus sp.* strain-B2 (HE805963). The terms of mortality vary between 12 and 22 days. This can be attributed to the mode of action of the majority of the genus *Bacillus* bacteria which act by releasing toxins that demand special conditions or by the conditions of the intestinal environment of crickets that do not fit the growth and bacterial multiplication which requires a relatively long time adaptation (Greathead *et al.,* 1994; Lacey *et al.,* 2001). Indeed, the use of *B. subtilis, B. thuringiensis* and *B. larvae* against larvae *Schistocerca gregaria,* showed 90% mortality, 80% and 70% achieved after 16 days in L4. However, *Pseudomonas aeruginosa* has caused total mortality after 4 days (Mohand-Kaci & Doumandji-Mitiche, 2006).

Finally, in a recent study, the effects of acute toxicity from topical application of growth regulators on three *Locusta migratoria var. manilensis* were evaluated in laboratory conditions with a recorded mortality rate of 97% for flufenoxuron, 100% for azadirachtin and 48% for pyriproxyfen (Bi Zhen et *al*., 2012).

Calculation of TL50 and LC50

Thus, the lethal time for 50% of individuals varies on the bacteria and the bacterial concentrations administered (Figure 4). The L5 larvae of *L. migratoria* processed by *Bacillus sp.* strain-B1 (HE799656) presented the lowest LT50 with a value of 3.32 days recorded at the high dose, 9.12 days for the middle dose and 13.37 days for the low dose. They are followed by L5 larvae treated by *Bacillus sp*. Strain-B2 (HE805963) with 9.19 days for the high dose, 12.94 days for the intermediate dose and13.87 days for the low dose.

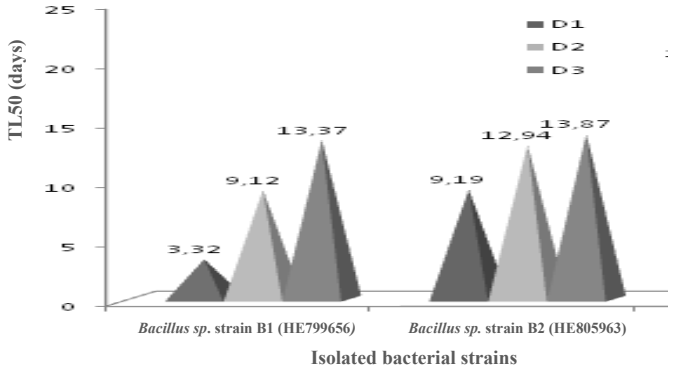

Figure 4: TL50 obtained for L5 larvae of *L. migratoria* treated with 3 concentrations of *Bacillus sp. Strain-B1* (HE799656) and *Bacillus sp. Strain-B2* (HE805963).

The comparison of LC50 obtained for mortality observation time for 5, 7 and 14 days after treatment shows that the LC50 is closely related with time; it varies depending on the tested bacterial strain (Figure 5). Indeed, the lowest LC50 are obtained at the 14th day with 0,37mg/ ml and0.62 mg/ ml for the strains *Bacillus sp.* strain-B1 (HE799656) and *Bacillus sp.* strain-B2 (HE805963). The higher LC50 are recorded on the 5th day with values of 1.37 and 2.87 mg/ml.

Furthermore, if one compares the lethal dose by ingestion of the different tested strains, we can see that the strain *Bacillus sp*. Strain-B1 (HE799656) shows considerably more effective and fast reaction than others. It presents the LC50, the lowest in all tested times (5, 7and 14 days after treatment).

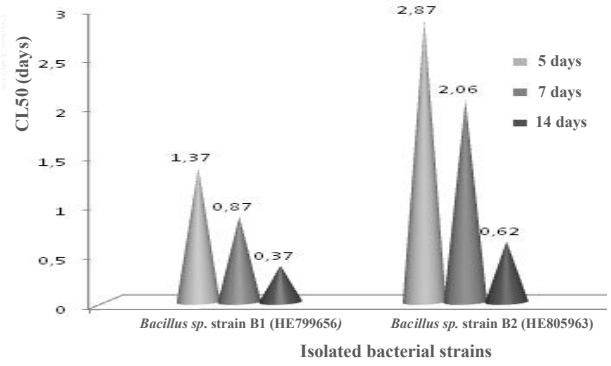

Figure 5: CL_{50} obtained for L5 larvae of *L. migratoria* treated with 3 concentrations of *Bacillus sp.* strain-B1 (HE799656) and *Bacillus sp.* strain-B2 (HE805963).

Our results agree with those of Ould El Hadj et *al*. (2006) which recorded a 7,5 days TL50 for L5 larvae *Schistocerca gregaria treated* with Neem, 8.2 days to 10.4 days for *Melia* and *Eucalyptus*. In addition, comparison of the sensitivity of *Locusta migratoria* to a commercial product and a local strain of *Metarhizium* showed that the latter has a higher activity with a pathogenic TL50 of 7.1 days (Niassy et *al*., 2011).

Gry *et al. (*1966) notes that the calculation of lethal doses determines the dose of the insecticide to be applied to the insect to obtain the desired percentage of mortality, allowing to judge accurately the insecticide product's power. The mortality rate in adult females of the mite *Tetranychus urticae* increased with increasing the concentration of conidia of two strains of entomopathogenic fungi and the most virulent against the mite was *Paecilomycis fumosoroseus,* having the lowest value of LC50 (9.1×10^4 conidia/ml) and LT50 (4.58 days to 1×10^8 conidia/ml) followed by *Verticillium lecanii* with an LC50 (1.7×10^6 conidia / ml) and LT50 5.45 days 1×10^8 conidia / ml (Amjad et *al*., 2012).

Conclusion

In conclusion, despite the proven value of these biological control agents, it appears that they are not much used. Yet they seem to offer the best prospects for biological controls, especially those that can be formulated to be multiplied and spread like biopesticides, particularly during locust control campaigns where they help reducing the outbreak mass.

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EVALUATION OF METAL AND METALLOID CONTAMINATION IN SOIL FROM MINING WASTE FOR REHABILITATION AND FUTURE LAND USE

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Abstract

Following mining of ore, extraction or separation of the economic item is required. Mine site wastes comprising waste rock and tailings are held in separately constructed structures to minimise erosion and contaminant transport. Key contaminants present in waste from base metal mining and other sulfidic deposits are metals (e.g. cadmium, copper, lead and zinc) and metalloids (antimony, arsenic, bismuth and selenium). Because processed mine minerals are finely divided, there is potential risk from such materials entering the environment and food chain and to be transferred to animals/humans such as via cattle grazing. Principle 4 of the International Council on Mining and Minerals ten principles for sustainable development of mining identifies quantitative indicators of rehabilitation success to: ‘implement risk management strategies based on valid data and sound science’. To avoid health risks to communities from mining activities, good prediction and planning is required, together with well-designed monitoring to detect adverse trends of contaminants. A risk assessment process underlies the risk management strategies adopted. The bioavailability of contaminants is a key component but data is generally lacking to provide a realistic health risk assessment of arsenic and metals from mine wastes. This paper provides an overview of the rationale and application of using metal and metalloid bioavailability data for risk assessment of rehabilitated mined land and its application to develop site specific guidelines for protection of human health and the environment and potential of metal uptake by grazing animals.

Keywords: *Metals/metalloids; mining; contaminants; risk management; bioavailability.*

Introduction

In mineral rich countries such as Australia, mine sites are potential sources of metals and metalloids. If not properly managed, mining activities and dispersion of such contaminants may cause adverse effects on the environment and human health. Limiting the effects of mineral processing activities on nearby communities is vital for sustainable development in mining and mineral processing to avoid environmental and human health impacts from associated contaminants (ICMM, 2007). The International Council on Mining and Minerals (ICMM, 2003) developed ten principles for sustainable development in the mining and minerals industry. These principles have been adopted in Australia and elsewhere to promote rigorous mine closure programs before mining completion and identify quantitative indicators of rehabilitation success. In particular, Principle 4 of the ICMM’s ten principles describes ‘implementing risk management strategies based on valid data and sound science’(MCA, 2005). Often, high costs are incurred during the mine rehabilitation process in an effort to return the land to its former state and/or an accepted future land use. A common option in Australia is the utilisation of mined land for future pastoral activity. Criteria may also be developed to assess the risk to grazing animals, from arsenic and lead liberated from rehabilitated mine wastes (Bruce, 2004; Ng et al., 2014).

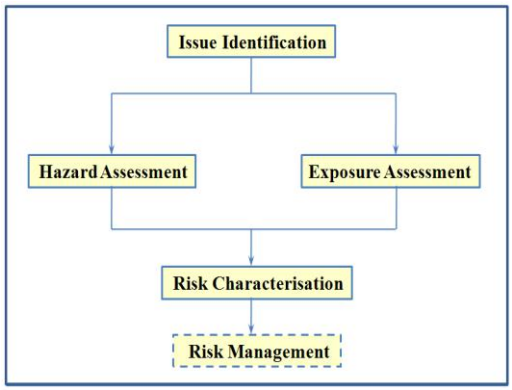

Figure 1. Framework for human health risk assessment (enHealth, 2012)

The Australian National Environmental Protection Council (NEPC, 2013) (Figure1) set Health Investigation Levels (HILs) for site contaminants in soil including some metals and arsenic (Table 1) based on guiding principles for health risk assessment (enHealth, 2012). Although no specific HILs exist for mine sites in the current Australian National Environmental Protection Measure (NEPM) (NEPC, 2013), the enHealth risk assessment framework is universally applicable to mine site health risk assessment. Bioavailability data is generally lacking for realistic health risk assessment of metals and metalloids from mine tailings in Australian conditions (Diacomanolis et al., 2007). If the health-based investigation level (HIL) is exceeded (Table 1), a further Tier II risk assessment of the site is required. In the absence of site specific data, the HILs may not provide accurate close out criteria for mined land (Ng et al., 2003). Significantly, the revised Australian NEPM (NEPC, 2013) adopted the concept of bioavailability and this can be considered in a “Tier-two” risk assessment step. Bioavailability (in-vivo) is defined as the fraction of a compound that is absorbed and reaches the circulatory system after ingestion, inhalation or dermal contact (Ng et al., 2015). Bioaccessibility (in-vitro) is the proportion of metal and metalloid that can be absorbed by an organism and is generally a more conservative approach to predict bioavailability of contaminated wastes such as from mining.

Table 1. Soil contamination health investigation levels in Australia (NEPC, 2013)

Metal or metalloid (mg/kg)	Australian soil contamination Health Investigation Levels (NEPC, 2013)			
	Residential A (Low-density with garden)	Residential B (High-density minor garden)	Residential C (Open-space recreational)	Residential D (Commercial/industrial)
Arsenic	100	500	300	3,000
Cadmium	20	150	90	900
Copper	6,000	30,000	17,000	240,000
Lead	300-	1,200	600	1,500
Zinc	7,400	60,000	30,000	400,000

The bioavailability of a contaminant is a key component of a risk assessment and is conservatively assumed to be 100% when no specific data exists. Research to generate bioavailability data for various metals and metalloids of mine tailings has been undertaken in order to provide a more accurate risk assessment of rehabilitated mine facilities under Australian conditions and to develop quantitative indicators for mine closure (Bruce et al., 2002; Diacomanolis et al., 2010). In-vivo bioavailability of arsenic from contaminated land has been assessed using small mammals including rodents, meadow voles, dogs, pigs, monkeys and more typically guinea pigs or rabbits (NRC, 2003). The rat model was utilised

to estimate arsenic and lead bioavailability to provide a risk assessment in calculating the potential exposure route via soil ingestion (Diacomanolis et al., 2007). Absolute bioavailability is measured via animal uptake but is expensive and time consuming. A more practical approach uses in-vitro PBET (physiologically based extraction test) to determine the bioaccessibility of metals and metalloids in soils (Bruce et al., 2007; Ruby et al., 1996).
When mined land is utilised for pastoral activity the key component of risk assessment is the ability to estimate a realistic and safe exposure, or dose rate, of the mine tailings to the grazing cattle over a set period of time. Grazing trials conducted directly on rehabilitated tailings facilities have provided valuable in-situ information on arsenic and heavy metal dose rates and associated accumulations (Bruce et al., 2002).
A review of current bioaccessibility methods and their limitations gives guidance in the Australian NEPM for determining their use and application in contaminant site assessment specifically of arsenic and heavy metals to give prediction of bioavailability (Ng et al., 2015). Comparison of in-vivo bioavailability measurement using animal (rat) uptake provides a means to validate the in-vitro bioaccessibility measurement (Bruce et al., 2007; Ruby et al., 1996).
This paper reviews the significance of speciation and bioavailability of arsenic and metals in managing health risks for mine site rehabilitation and shows how mine site rehabilitation can be better managed by mapping contamination with bioaccessibility-adjusted concentration data. Site specific guidelines can then be established based on the bioaccessibility and bioavailability assessments undertaken for arsenic and lead.

Materials and methods

Two different approaches were employed as risk assessment tools: (i) the in-vitro PBET (physiologically based extraction test) determination of bioaccessibility of individual soils (Bruce et al., 2007; Ruby et al., 2006); and (ii) in-vivo bioavailability measurement of composite wastes using rats (Diacomanolis et al., 2007). All mine waste samples were tested for bioaccessibility (PBET) in a synthetic gastric fluid system under increasing pH values of 1.3, 2.5 and 4.0 simulating fasting, partially fed and fed stomach conditions respectively, and at pH 7.0 simulating the small intestinal pH condition for the extraction tests (Bruce et al., 2007; Ruby et al., 1996). The case study of the Woodcutters lead zinc mine in northern Australia was used to illustrate site specific guideline development for arsenic and metals in soil (Diacomanolis et al., 2010) from on-site contamination. Woodcutters ore production commenced as an open pit in 1985 and became an underground operation in 1986. Mining ceased in March 1999; the mine closure program for the site has continued since then.
A metal and metalloid survey of 60 surface soils was conducted at Woodcutters mine in September 2005; the soil samples were representative of 4 different categories of mine waste materials (Table 3). Finely ground soils (<70 μm) were digested in aqua regia and total arsenic and metal concentrations determined by ICP-AES.
In-vivo bioavailability testing on composite samples of mine wastes from Categories 1-4 was undertaken using animals. Rats weighted at approximately 180 g each were divided into groups of 4. For positive controls, rats were injected intravenously using the salt solution and the other groups were given slurries of mine material by oral gavage. The mine materials were weighted for each rat separately according to the dose rate and body weight. The rats were kept in individual metabolic cages and were fasted over the night before dosing. The dose rates were: arsenic 0.5 mg As/kg b.w. (in the form of sodium arsenate or sodium arsenite, the latter being most conservative) given intravenously via the tail vein for the positive control and lead at 1mg Pb/kg b.w. as lead acetate solution; other metals were not considered for this bioavailability assessment. Rat treatment groups were dosed by oral gavage with 1g of mine waste in a slurry per 200 g body weight. Urine samples were collected 24 h prior to dosing

and then daily over 10 days post dosing; arsenic and lead were determined in urine by ICP-MS. Bioavailability was calculated from the area under the urinary excretion curve of a treatment group compared to that of the positive control group. The complete data analysis is given elsewhere (Diacomanolis, 2013).

Results and discussion

Mean arsenic and metal concentrations in mine wastes are summarised in Table 2.

Table 2. Total concentrations (mean ± s.e.) of arsenic and metals in Woodcutters mine wastes

Element (mg/kg)	Category 1 Creek sediment	Category 2 Material processing	Category 3 Waste rock	Category 4 Tailings
Arsenic	180±50	1340±720	330±40	450±50
Cadmium	120±70	70±40	9±3	5±1
Copper	105±10	130±30	120±30	110±8
Lead	840±280	5450±2700	870±230	550±80
Zinc	7800±2400	7700±4400	1200±470	820±120

A desk top risk assessment on total arsenic and metal concentrations (Table 2) showed that many samples exceeded the HILs for Residential C-open space recreational use (Table 1). In the absence of site specific bioavailability and exposure data, a worst case scenario was necessary to identify potential “hot spots” based on the accepted risk assessment framework (enHealth, 2012). All mine waste samples were then tested for bioaccessibility using PBET. Mean percentages of bioaccessibility for arsenic, cadmium, copper, lead and zinc from the 4 categories of waste materials are shown in Table 3.

Historical investigations into the distribution and concentrations of metals at the Woodcutters mine site indicate that there was an elevated natural background of various minerals. Accordingly, site specific remediation guidelines were developed for Woodcutters mine site following the bioaccessibility studies. The approach adopted now follows the procedure established by the NEPC (2013) and led to the further refinement of the proposed remediation guidelines (Table 3). The measurement of % BAc using PBET was confirmed by rat bioavailability (%BA) of arsenic and lead in key soil types and enabled the development of the site specific remediation guidelines. Application of the site specific guidelines from the risk-based approach indicated that the extent of contamination was limited to a small number of discrete locations at the mine site. Arsenic showed good agreement between BA and BAc, while BAc for lead was approximately 10 times higher than for BA and indicated that BAc was a conservative measurement for lead (Table 3).

Table 3. Summary of bioavailability (BA) and bioaccessibility (BAc) data obtained in 2005 and proposed remediation guidelines for Woodcutters mine site rehabilitation

Metal/metalloid	% BAc 2005	% BA 2005	NEPM Residential C (mg/kg)	Proposed Remediation Guideline (mg/kg)
Arsenic	3-10	1.6-8.9	300	1000
Cadmium	17-30	-	90	80
Copper	5-13	-	17,000	10,000
Lead	10-18	0.6-1.4	600	1,500
Zinc	23-27	-	30,000	40,000

Although the 2005 soil survey results for total arsenic and lead concentrations were relatively high in the mine site rehabilitated areas, adjustment of the results using % BAc from PBET indicated that the contamination was not a significant health risk (Table 2). Only cadmium at

one site and lead at 4 sites in soil were considered to be contaminated and significant when compared against the site specific remediation criteria.

A second key component of arsenic and metal risk assessment is the estimation of realistic exposure, or dose rate, of the mine waste to the grazing cattle over a set period of time (not described here in detail). Hence, grazing trials can be conducted directly on rehabilitated tailings facilities and have provided valuable in situ information on arsenic and heavy metal dose rates and associated accumulation at other mine sites (Ng et al., 2014).

Conclusion

The investigation and development of site specific thresholds demonstrates that careful examination of specific source characteristics and receiving context can greatly improve the focusing and application of resources in closure processes. This had a significant bearing on the focus and extent of remediation activities and success of the Woodcutters mine closure process in enabling local people to access the site for recreational purposes.

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SIGNIFICANT ENVIRONMENTAL ISSUES FROM HERBICIDE AND PESTICIDE USE IN MULTIPLE AGRO-BASED ACTIVITIES IN NORTHEAST THAILAND

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Abstract

Extensive herbicide and pesticide application is undertaken for a diverse range of agro-based activities of the Namphong River sub catchment of the Mekong River in Northeast Thailand. The Namphong River has extensive in-river cage aquaculture for Tilapia production in addition to rice and mixed vegetable cultivation, pulp and paper manufacturing utilising local eucalyptus tree cultivation and sugar cane for molasses and ethanol production. The agro-based activities potentially add herbicides and pesticides to river flow and affect the aquatic ecosystem and food chain. Passive samplers were deployed at 5 sites along the Namphong River and used to accumulate herbicides and pesticides on polydimethyl siloxane (PDMS) strip during 29 days deployment in January-February 2012 (analysed at the Queensland Health Laboratories, Brisbane, Australia). Measured herbicides and pesticides except chlorpyrifos were present at exceedingly low concentrations in Namphong river water. However chlorpyrifos was <20 times lower than an Australian aquatic guideline (10 ng/L; 95% protection aquatic freshwater species for protection of aquatic species and was identified as the highest volume of insecticide importation in Thailand. Comparison with an earlier 2005 study of the paddy field ecosystem 50 km N from Khon Kaen shows a shift from organochlorine use to low persistence pesticides, particularly chlorpyrifos. Increased application rate of chlorpyrifos in the Namphong River may increase and exceed the threshold of toxicity for fish and other species and be a threat to cage aquaculture.

Keywords: *Herbicides; pesticide; passive sampling; land use practice; environmental risk management.*

Introduction

The Namphong River in the Northeast (NE) part of Thailand is within a sub-catchment of the Mekong River (Fig. 1). The Namphong River flows to the Chi River via the Mun River to the Mekong River. Below Ubolratana Dam, extensive agro-industry and farming activities along the Namphong River use irrigation from dam storage and some supplementary groundwater supply. Pesticides and herbicides from agro-industry activities are identified as potential risks to human health and the environment and as significant pollutants (Somparn et al., 2014). Chlorpyrifos was identified as the highest volume of pesticide importation in Thailand (Department of Agriculture, 2012). The aquatic ecosystem of the Namphong River is a critical aspect for evaluation of pesticides dispersed in the environment. Ecotoxicological effects from pesticides on aquatic organisms may be observed via biomonitoring with both individual organisms and ecosystem function and structure. The Namphong River also supports

extensive cage aquaculture which may be affected by pesticides. In 2010, 209 cages produced 1536 tonnes of Nile tilapia in Khon Kaen province (Department of Fisheries, 2010).
This study aims to identify if pesticides and herbicides from multiple agro-based activities comprising agro-industry and small-scale farming activities along the Namphong River, are environmental and health risks, causing any impact to the Namphong River water body at different sample locations, and compare with existing data from the 2012 dry season.

Materials and methods

Pesticides and herbicides were accumulated on polydimethylsiloxane (PDMS) strip passive samplers during 19 January-23 February 2012 at 5 sites at the Namphong River from below the Ubonratana Dam, the tributary downstream of the paper mill and vegetable and paddy fields down to the Chi River above the junction of Namphong River (Fig.1).

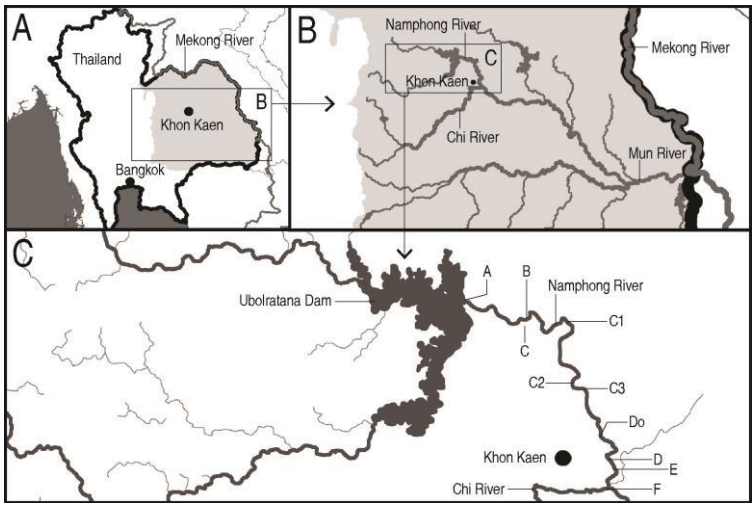

Fig. 1 Location of study site in the Mekong River basin NE Thailand (Maps A and B) and sampling sites along the Namphong River (Map C) *[Sites A – Ubolratana Dam; B – fish cage (in-river cage aquaculture for Tilapia production); C – pulp/paper industrial plant (discharge via small tributary to main river); sugar industrial plant; C2 cucumber culture; C3 corn culture; Do vegetables culture; D vegetable culture and paddy fields; E vegetables culture; and F – vegetables culture, residential discharge to Chi River just upstream from confluence with Namphong River]*

Site C is on a small tributary less than a few km to the Namphong River from the pulp and paper mill (Fig. 1.C). Prior to application each PDMS sampler (strips 2.5 cm x 92 cm and thickness about 400 μm) was cleaned on a horizontal shaker in fresh redistilled hexane for three consecutive 24 h periods followed by drying under high purity nitrogen gas steam, wrapping in acetone rinsed aluminium foil and stored in the refrigerator until dispatched to Khon Kaen. The PDMS samplers were placed under water at 0.50 m depth and left for 29 days. Following collection, the PDMS samplers were placed in pre-cleaned acetone rinsed aluminium foil envelopes and refrigerated until dispatched with quarantine clearance details from Khon Kaen to Australia for sample processing and analysis using the routine procedures of Queensland Health Organic Chemistry Laboratory. Prior to PDMS extraction in hexane and following instrumental analysis each of them was cleaned by scrubbing with water, dipping in redistilled hexane for 30 seconds and 0.5M HCL for 20 seconds followed by rinsing with acetone and isopropanol. Water quality measured using standard techniques described (Komarova et al., 2012, 2013).
Passive samplers using polydimethylsiloxane are low cost and have an improved affinity for polar compounds relative to other samplers (Rusina et al., 2007). Comparative studies (Shaw et al., 2010) showed that PDMS samplers accumulated many compounds with log Kow 2.9 to

6.4 dissolved in water. The mass captured was 6 times higher in PDMS compared with other samplers with the same surface area at log Kow of 4.7 (chlorpyrifos) and 4.27 (trifluralin).
Well developed and validated procedures are used at all steps of PDMS passive sampler application (Shaw, 2005). The mass of contaminants accumulated in a PDMS sampler are converted to their concentrations in water (Cw, ng/L) using a sampling rate (Rs in Ld^{-1}) that is estimated for different pesticides at different environmental conditions in laboratory calibration study (Huckins et al., 2002) by Eq.1:

$$Cw = M_{PDMS} / Rs\ t \quad (1)$$

where M_{PDMS} is the mass of a pesticide found in the PDMS sampler after deployment (ng) and t is the deployment time in days. Method detection limits are calculated from the lowest mass per sampler detected on analytical equipment (LCMS, GCMS) and respective compound sampling rate (e.g. <50 ng/mL for the extracted volume in the laboratory and 0.001 ng/L for chlorpyrifos in PDMS samplers for 29 day deployment). The detection limits are an order of magnitude lower than for pesticide concentrations using grab sampling techniques (Shaw, 2005). The linearity of the relationship between log Kow and sampler to water partition coefficients covers a wide range of compounds for PDMS (Yates et al., 2007).
After extraction and extensive clean-up procedures using size exclusion chromatography, pesticide and herbicide are analysed by different instrumental analytical methods: these are gas chromatography (GC), high performance liquid chromatography (HPLC), GC/MS and LC/MS-MS undertaken in the Queensland Health NATA accredited laboratory (according to ISO 17025) and based on USEPA Method EPA 503/6-90-004. All standards (3 external standards) and samples are spiked with an internal standard just prior to analysis.

Results and discussion

Table 1 gives the pesticide and herbicide concentrations with highest inputs for effluent at Site C (Paper Mill) when compared with Table 2. Galaxolide, terbuthylazine, phosphate tri-n-butyl, tonalid and terbutryn are added in paper mill effluent (Table 1) but only galaxolide remains detectable at much lower concentration (1:35) showing dilution down to Site F at the Chi River; tonalid is diluted 1:10 at Site C3 (Fig. 1.C) but is then undetectable downstream.
Table 2 gives the pesticide and herbicide concentrations for all sites upstream or lower downstream; i.e. below Site C2. Ametryn, oxadiazon, chlorpyrifos, prometryn and atrazine are all found at measureable concentrations at Site A below Ubonratana Dam (Fig. 1.C) indicating that application of these pesticides and herbicides has occurred in the upper Namphong River catchment above Ubonratana dam and contribute to the downstream river load. Fig. 1 shows that about 60% of the Namphong River catchment is associated with the two branches above Ubonratana dam. The input of ametryn, oxadiazon, chlorpyrifos, prometryn and atrazine increased from Site 3 downstream (Fig. 1.C, Table 2). The comparison of measured concentrations of pesticides and herbicides for all sites in Tables 1 and 2 with compiled details of guidelines and LC50 values for fish and other freshwater species shows that all compounds are well below concentrations that will induce a toxic response. While all compounds excepting chlorpyrifos are present at exceedingly low concentrations, chlorpyrifos is only 10-20 times lower than the validated Australian aquatic guideline ANZECC/ARMCANZ (2000) (Table 2 – note no Thai guideline available).
The application of chlorpyrifos is associated with insect control of vegetable and paddy field areas in line with the highest volume of insecticide importation in Thailand cited above (Department of Agriculture, 2012). The pesticides and herbicides that were detected at the sampling sites of the Namphong River in January 2012 are all low persistent compounds and indicate recent applications. Comparison of the observed pesticides and herbicides identified in this study with an earlier 2005 study of paddy fields, horticulture and vegetable farming in the Namphong River basin sediment, water samples and PDMS samplers (Boonthai-Iwai et

al., 2007) showed that the pesticides and herbicides in use at that time included atrazine, oxadiazon and the organochlorines dicofol and endosulfan. Tables 1 and 2 now show there was no detection of organochlorines in 2012 but that chlorpyrifos is used extensively together with a range of herbicides. If the application rate of chlorpyrifos increases, the level in Namphong River may increase and exceed the threshold of toxicity for fish and be a threat to cage aquaculture. Thus there needs to be a more systematic and complex assessment of chlorpyrifos in the Namphong River basin to develop a tool for its control and environmental risk management.

Table 1. Pesticide and herbicide concentrations for 19-23 January 2012 showing Site C (Paper Mill) effluent with highest input (average of 2 PDMSs per site)

Sampling site	Galaxolide[a]	Terbuthylazine	Terbuphos	Phosphate Tri-n-Butyl	Tonalid	Terbutryn
A. Ubolratana (ng/L)	0.201	<0.001	<0.001	<0.001	<0.001	0.069
C. Pulp mill (ng/L)	26.8	3.1	1.1	0.18	0.17	0.34
C3. Corn (ng/L)	0.68	<0.001	<0.001	<0.001	0.017	<0.001
D. Vegetable/ paddy field (ng/L)	0.81	<0.001	<0.001	<0.001	<0.001	<0.001
F. Vegetable (ng/L)	0.89	<0.001	<0.001	<0.001	<0.001	<0.001
Guideline or acute toxicity						
Algae, protozoa LC50 (ng/L) (WHO, 1991)	-	-	-	3.2-100 x 10^6	-	-
Fish LC50 (ng/L) (PAN, 2014)	-	0.16-90 x 10^6	2340 – 20000	-	-	0.8 – 10.0 x 10^6
Fish LC50 (ng/L) (PFW Aroma Chemicals, 2007)	-	-	-	-	0.318 x 10^6	-

Note: a. Not toxic to aquatic species, with low toxicity to terrestrial species [Rat Acute: >5000 mg/kg rat (Spectrum, 2009)]

Table 2. Pesticide and herbicide concentrations for 19-23 January 2012 showing inputs at all sites upstream or below Site C downstream (average of 2 PDMSs per site)

Sampling site	Ametryn	Oxadiazon	Chlorpyrifos	Prometryn	Atrazine	Trifluralin
A. Ubolratana (ng/L)	2.6	0.46	0.061	0.23	0.10	<0.001
C. Pulp mill (ng/L)	0.12	0.75	0.24	<0.001	0.10	<0.001
C3. Corn (ng/L)	1.7	1.0	0.45	0.14	0.29	<0.001
D. Vegetable/ paddy field (ng/L)	2.7	6.7	0.79	0.30	0.40	0.062
F. Vegetable (ng/L)	2.6	7.2	0.88	0.28	0.40	0.18
Guideline or acute toxicity						
95% protection aquatic freshwater species (ANZECC/ARMCANZ, 2000) (ng/L)	-	-	10	-	13000	4400
Nile tilapia LC50 (ng/L) (PAN, 2014)	-	60000	-	-	-	-
Fish LC50 (ng/L) (EXTOXNET, 2014a,b)	3.2 – 8.8 x 10^6	-	-	2.5 – 10.0 x 10^6	-	-

Conclusion

This paper identified that the Namphong River basin can absorb current inputs of pesticides and herbicides that arise from the agro-based activities and is not causing any impact to the water quality in the Namphong River itself. Measured pesticides and herbicides except chlorpyrifos were present at exceedingly low concentrations in Namphong river water. However chlorpyrifos is only 10-20 times lower than the Australian aquatic guideline (noting no Thai guideline) for protection of aquatic species and was identified as the highest volume of insecticide importation in Thailand. If the application rate of chlorpyrifos is increased, the level in Namphong River may increase and exceed the threshold of toxicity for fish and other species and be a threat to cage aquaculture. Although there is no current contamination problem there is a potential issue with chlorpyrifos in water which has higher risk and requiring further data and information. Thus more detailed assessment of chlorpyrifos in the Namphong River basin is required to improve control and environmental risk management.

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SEASONAL DYNAMIC ANALYSIS OF AEROALERGENIC POLEN OF BIRCH, ALDER AND HAZEL IN BANJA LUKA (B&H) DURING 2008-2014

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Abstract

Members of the subclasses *Hamamelidae* are characterized by the production of huge quantities of pollen grains transmitted by air. Among them an important source of allergens represents pollen of birch, alder and hazel, as the most frequent causes of pollen allergies in the spring. Considering that the mentioned species are characterized by moderate to strong, or very strong allergenic capacity in the case of birch, the main objective of this study was to monitor the seasonal dynamics and analyze 7-year monitoring of mentioned allergenic species in Banja Luka.

Systematic measurements are carried out using the method defined by the International Association for Aerobiology (IAA).

During the 7-year period, for pollen representatives of the family *Betulaceae,* it is determined birch pollen domination and considerably lower proportion of total alder and hazel pollen count in Banja Luka (Bosnia and Herzegovina). On annually level maximum total number of p/m^3 for *Alnus glutinosa* was registered in 2014 (426 p/m^3). Same year, the maximum total number of 311 p/m^3 was registered for hazel, while for birch the maximum total number of 1223 p/m^3 was registered in 2011.

Keywords: *seasonal dynamics, pollen aeroallergens, Betulaceae, Banja Luka*

Introduction

Members of the subclasses *Hamamelidae* (Cronquist, 1988) are characterized by small and often unisexual flowers without leaves, as well as the production of huge quantities of pollen grains transmitted by air (so-called anemophiles species). Such case is with the species of order Fagales (family *Betulaceae: Alnus* spp., *Betula* spp. and *Corylus* spp.) which presents a significant source of allergens in northern, central and eastern Europe (Łukasz *et al*., 2014). Alder, hazel and birch are actually among the earliest flowering plants and in which beginning of pollen release depends on the atmospheric conditions, especially from the cumulative air temperature (above zero) (Scamoni, 1955). The intensity of pollen release and total count of pollen grains are also affected by the meteorological conditions in the previous year (August), when the sporogennous tissue begins to develop (Rodkiewicz *et al*., 1996). *Corylus* and *Alnus* reacting as primers of allergic sensitisation to *Betulaceae* pollen allergens, resulting in greater clinical symptoms during the *Betula* pollen season (Ianovici *et al*., 2009). It has been pointed out that *Betula* pollen concentrations greater than 30 pollen grains/m^3/day trigger severe symptoms, and values greater than 80 pollen grains/m^3/day produce symptoms in 90% of patients (Corsico, 1993). As well according to Vik *et al*. (1991) *Corylus* pollen gives allergic reaction at concentrations of 20:30 pollen grains/m^3 and *Alnus* pollen at concentrations of 50 pollen grains/m^3.

Considering the above and that the species in the family *Betulaceae Corylus avellana* - hazel and *Alnus glutinosa* - alder are characterized by moderate to strong allergenic properties and *Betula pendula* - birch are characterized by very strong allergenic properties main objective of this study was to monitor the seasonal dynamics of allergenic species, or determined the beginning, duration and end of the period of pollination. The obtained data are presented

through the media of public information in the form of daily aeropalinology reports and forecasts of the seven-day period.

Material and method

Monitoring the concentration of aeroallergenic species of alder pollen (*Alnus glutinosa*), hazel pollen *(Corylus avellana*) and birch pollen *(Betula pendula*) during the period of pollination (febraury – may from 2008 to 2014) was conducted at the Public institution Agricultural Institute of Republic of Srpska, Banja Luka (PI AIRS, Banja Luka) (N 44º47`41.0``, E 017º12`22.6``), by Hirst's type pollenometar (Hirst, 1952) using the method defined by the International Association for Aerobiology (IAA). The device Burkard (Burkard Manufacturing Co., Uxbridge, Middlesex, UK) is calibrated for sampling 10 liters of air/min through a hole 14 x 2 mm diamtear, which always faces in the direction of the wind and it is protected from direct rainfall. By passing, air through the hole, it comes to the fixation of pollen grains on glass coated with silicone gel, which is moving at a speed of 2 mm/h.

Visual identification, or qualitative and quntitative assessment, of sampled pollen grains was carried out on a daily basis after 24 hours, based on the morphological characteristics of species in the family *Betulaceae* under a light microscope Olympus BX51 at magnification x400, according the International Association for Aerobiology and converting the obtained results in the concentration of pollen grains per m^3 of air. Immediately prior to screening, microscopic slide with 24-hour segment, is prepared by placing polyvinyl alcohol substrate (Gelvatol), phenol, and glycerol, which allows color fuchsin staining of pollen grains and easy separation of the same from dust particles and fungy spores. After preparation and drying microscopic slide determining the number of pollen grains is carried out by the method of longitudinal lines in two-hour intervals and reviewing the 3 horizontal lines. At the end of the analysis, the obtained values are converted to daily concentrations determined by multiplying the number of pollen grains by a factor F, depending on the characteristics of the device for sampling air, surface of 24 hour segment, characteristics of the microscope and the surface of the inspected sub-sample. Concentration of the pollen grains per m^3 of air is important for symptoms occurence of allergic reactions, because the number of pollen grains in 1 m^3 of air, which causes interference in most patients is not the same for all types of pollen. In case of alder pollen it is enough > 50 pollen grains per m^3 of air, the birch pollen > 30 pollen grains per m^3 of air while in acse the of hazel pollen it is enough > 12 pollen grains per m^3 of air to cause an allergic reaction.

In addition to daily concentrations during the sampling of *Betulaceae* aeroallergenic pollen it was determined seasonal dynamics of pollination species *Betula, Alnus* and *Corylus*, or beginning and end of the season with the maximum concentration values present aeroallergens in the area of Banja Luka.

Obtained data are presented to the public media in the form of aeropalynological reports or so called pollen semephore (tab. 1).

Tab. 1. *Number of trees pollen grains in the air with the corresponding percentage of people with a possible symptoms occurence of allergic reactions (Forsyth County Environmental Affairs Department Pollen Rating Scale, PRS)*

Pollen value	**No of pollen grains/m^3 air**	**Symptoms occurence of allergic reactions**
	trees	
Not present	0	No simptoms
Low	1-15	Only in extremely sensitive individuals
Medium	16-90	In 50% sensitive individuals
High	91-1500	Almost all allergic individuals
Very high	>1500	All allergic individuals

Results and discussion

Beginning of season pollination of aeroalergenic species of family *Betulaceae* (*Alnus glutinosa, Betula pendula and Corylus avellana*) in Banja Luka, during the seven years of monitoring, has been recorded since the second week of March for the period 2008 - 2011 and from the third decade of February for the period 2012 - 2014. According to the literature in Italy (Tedeschini *et al*., 2003), the beginning of pollination in plants that bloom in winter, like *Corylus* may occur later, which is exactly the case in the area of Banja Luka during 2008 - 2011, while earlier pollination of hazel was recorded during the three-year period 2012 - 2014. On the other hand for the species *Alnus glutinosa* and *Betula pendula* in the area of Banja Luka during the seven-year monitoring was recorded early pollination, from the end of the first decade and the beginning of the second decade of March, which also states in areas of northern Europe (Van Vliet *et al*., 2002). However occurence of alder and birch pollen grains is characterized by the appearance of actually low concentrations or absence of pollen grains from the beginning of pollination, which means that the allergic reaction could occur only in extremely sensitive individuals (Photo 1-7).

According to the monitoring more than 70% of pollen species in the family *Betulaceae* were sampled during March and April. However pollination season through seven years monitoring lasted until the third decade of May, which is actually longer pollination season for *Betulaceae* aeroallergen, and in such circumstances, or during long periods of pollination the risk of cross reactions between *Betulaceae* pollen and pollen of fruit species could increase (Radisic *et al*., 2003).

On anually level total recorded *Alnus glutinosa* pollen number/m^3 was 315 p/m^3 (2008), 294 p/m^3 (2009), 261 p/m^3 (2010), 252 p/m^3 (2011), 208 p/m^3 (2012), 359 p/m^3 (2013) and 426 p/m^3 (2014). Total recorded *Betula pendula* pollen number /m^3 was 1006 p/m^3 (2008), 957 p/m^3 (2009), 951 p/m^3 (2010), 1223 p/m^3 (2011), 703 p/m^3 (2012), 982 p/m^3 (2013) and 457 p/m^3 (2014). In cas of *Corylus avelana* total recorded pollen number /m^3 was 126 p/m^3 (2008), 118 p/m^3 (2009), 108 p/m^3 (2010), 100 p/m^3 (2011), 168 p/m^3 (2012), 130 p/m^3 (2013) and 311 p/m^3 (2014) (Photo 8).

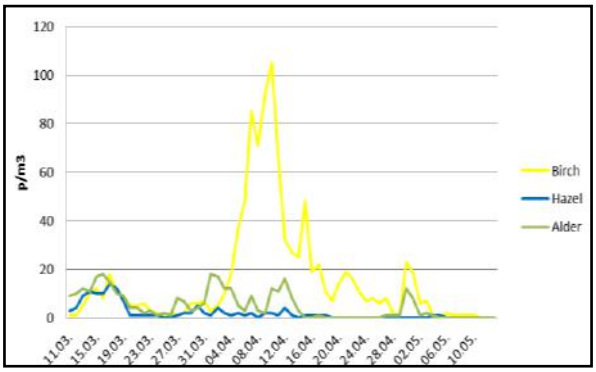

Photo. 1. *Seasonal dynamic of Betulaceae species pollination in 2008*

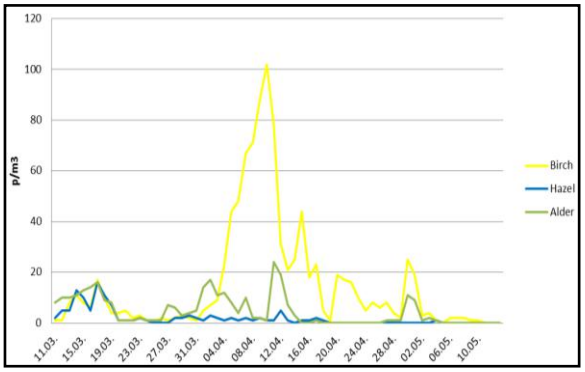

Photo. 2. *Seasonal dynamic of Betulaceae species pollination in 2009*

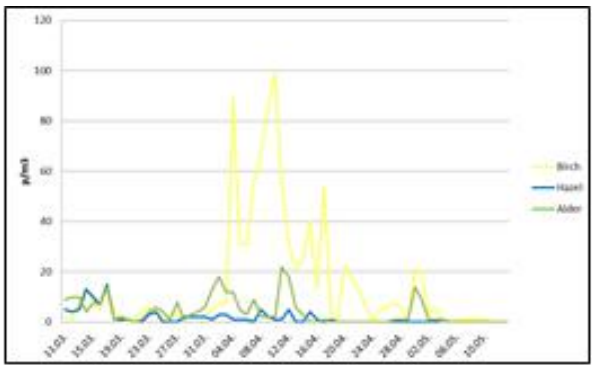

Photo. 3. *Seasonal dynamic of Betulaceae species pollination in 2010*

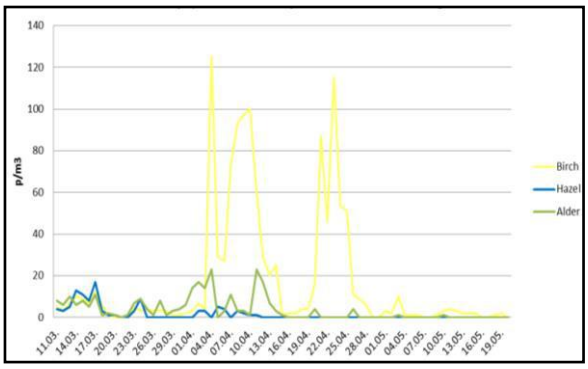

Photo. 4. *Seasonal dynamic of Betulaceae species pollination in 2011*

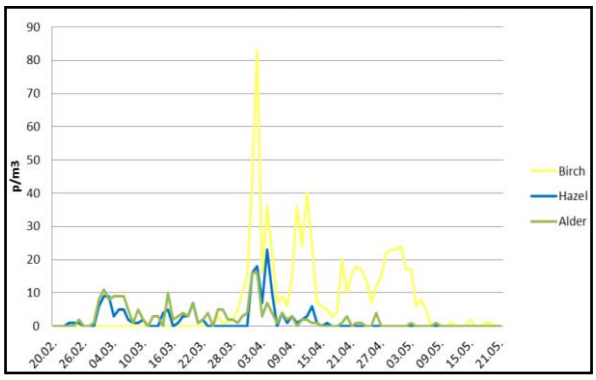

Photo. 5. *Seasonal dynamic of Betulaceae species pollination in 2012*

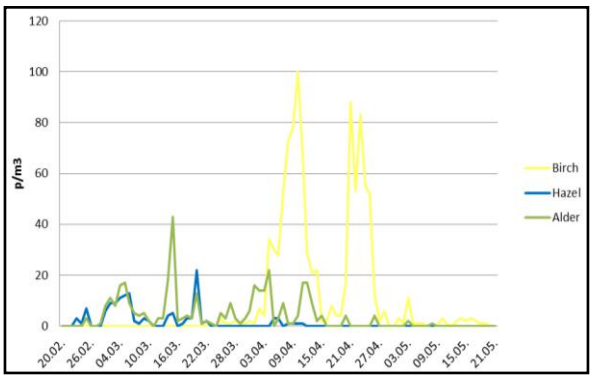

Photo. 6. *Seasonal dynamic of Betulaceae species pollination in 2013*

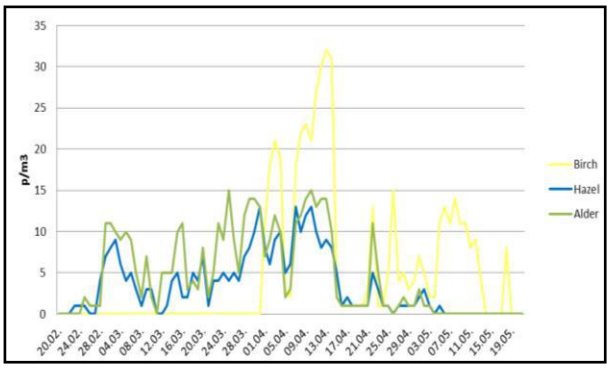

Photo. 7. *Seasonal dynamic of Betulaceae species pollination in 2014*

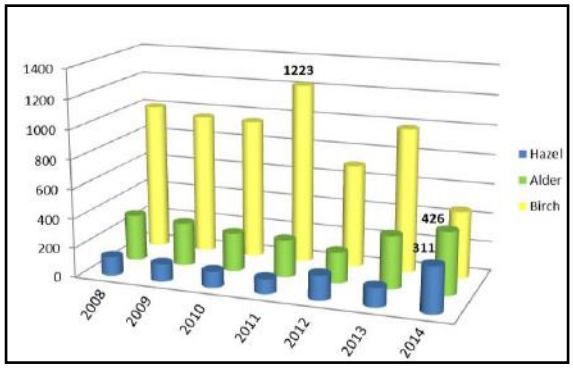

Photo. 8. *Concentration of Betulaceae pollen species in Banja Luka during 2008 - 2014*

Conclusion

During seven year monitoring (2008 - 2014) pollen of three species in the family *Betulaceae*, it was established dominance of birch pollen and proportionally significantly lower total recorded hazel and alder pollen value in the Banja Luka city. On the dominance of birch pollen indicates the number of days with a concentration of pollen that exceeds the level of occurrence of allergic reactions because the birch pollen ranged between 5-12 days, 2-4 days for the hazel pollen, while in case of alder pollen in seven monitoring was not determined a concentration of pollen that exceeds the level of allergic reactions occurrence.

In addition to birch pollen dominance among species in the family *Betulaceae*, according to results of monitoring the birch pollen season has shown significant variation in terms of the total amount of pollen on annualy level and the dynamics during the period of pollination, which is why monitoring is still necessary, especially for people with high sensitivity to *Betulaceae* species.

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OCCURENCE AND DISTRIBUTION MAPPING OF INVASIVE WEED SPECIES HELIANTHUS TUBEROSUS L. IN NORTH WESTERN AREA OF REPUBLIC OF SRPSKA

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Abstract

Species *Helianthus tuberosus* L., as many belonging to the *Helianthus* genus, are caracterised by their successful vegetative propagation, allelopathy or shading, aggressive spreading, elimination of other species of the natural habitats, changing the structure of plant communities, and even behave as dangerous weeds. Considering species habitat requirements, Republic of Srpska is definitely one of the area for potential distribution of Jerusalem artichoke as invasive weed species. In this sense main goal of this study was to investigate the occurence and carry out distribution mapping of Jerusalem artichoke in north western part of Republic of Srpska (RS).

Distribution and abundance parameters were done for 40 Helianthus stands based on Blanque Braun (1964) method and GIS software. A wide-spread distribituion reveal a high spreading and renewal potential of the species which turns it into a real threat to native flora and wildlife habitats, watercourses etc.

By current research in the north western part of RS Jerusalem artichoke is in significant expansion, resulting in negative ecological, economic and social impact. Regarding to those research priorization process for mentonied area on local level is of a great importance.

Key words: *Jerusalem artichoke, occurence, distribution, mapping, Republic of Srpska*

Introduction

The Jerusalem artichoke (*Helianthus tuberosus* L.), native to temperate regions of North America, has been grown in Europe since the 17th century (Slimestad *et al*., 2010) mainly for tubers. In the mid-18th century it was widely replaced by the potato as a staple food in central Europe (Hartmann *et al*., 1995). But this species, as many belonging to the *Helianthus* genus, are caracterised by their successful vegetative propagation, allelopathy or shading, they spread aggressively, eliminate other species of the natural habitats, change the structure of plant communities, and even behave as dangerous weeds. Another major issue related to the invasive taxa of this genus is that by their getting wild, they earn phenologic plasticity, probably hybridize, so their taxonomic position is uncertain and largely debated (Balogh, 2006). The first escaped plants were found in the mid 19th century in some countries, the invasive spread began mostly around 1900 and became more rapid in central Europe in the 1930s (Hartmann *et al*., 1995). Thus in many European countries *H. tuberosus* allready have invasive status or it was placed on watch-list wich comprises invasive alien species that have potential to cause damage and therefore their spread should be monitored (photo 1, http://www.cabi.org/isc/datasheet/26716).

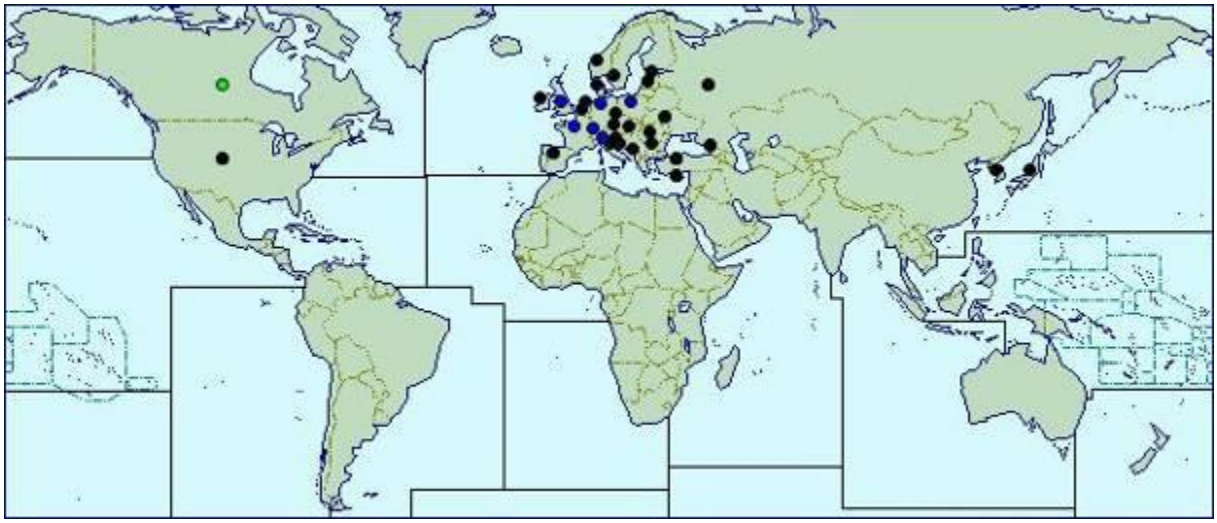

Photo 1. Naturalised distribution of Jerusalem artichoke: ●= Present, no further details; ●= Widespread; ●= Localised; ●=Confined and subject to quarantine; ●= Occasional or few reports; ● = Evidence of pathogen; ●= Last reported; ●= Presence unconfirmed; ●= See regional map for distribution within the country (source http://www.cabi.org/isc/datasheet/26716)

According to EPPO (European and Mediterranean Plant Protection Organization) the Jerusalem artichoke (*H. tuberosus* L.) is one of the 34 invasive taxa (source www.eppo.org/QUARANTINE/ias_plants.htm). The species is invasive even in its native country, disturbing natural forest communities (Balogh, 2006). It is considered an invasive species in Poland, Austria, Italy, Germany, France and Hungary (Wittenberg, 2005). According to Nature protection and biodiversity - State and impacts (Bosnia and Herzegovina) (Eropean Enviroment Agency) from 2010 the same status Jerusalem artichoke has in BiH. Among the invasive weed species *Helianthus tuberosus,* is one the most common in our area. This species also have a negative effect on the habitats of hygrophilous woods of willow, alder and poplar, which are particularly common in the Posavina area of Bosnia and Herzegovina (source www.eea.europa.eu/soer/countries/ba/nature-protection-and-biodiversity-state).

In spreading of invasive species one of the aggravating circumstances are floods. According to Fehér (2001), the floods also distort riparian vegetation and thus creating new opportunities for colonization by invasive species and forming a source population for spreading invasive species into the surrounding landscape. As an invasive species on river banks, *H. tuberosus* can result in increased flood erosion, as it has fewer fine roots than native river bank vegetation and leaves the soil bare and unprotected after it dies back in autumn. In addition, its tubers are dug after by rodents resulting in further damage to river banks and flood protection dams (Hartmann *et al.*, 1995; Kowarik, 2003). Regarding to those research in Rebublic of Srpska, with already conolized area by invasive Jerusalem artichoke, flooding waves recorded during 2010 and 2014 are aditional threath for further spreading.

Spreading potential, aggressiveness and invasiveness of Jerusalem artichoke as invasive weed species, as well as all facts mentonied above were the background to the main goal of this study which was to evaluate the occurrence, as well as to carry out distribution mapping of invasive species *Helianthus tuberosus* in north western part of RS.

Material and method

The current research mainly focuses on the north western part of RS in relation to species habitat requirements and main environmental features. Field survey was conducted during the vegetation period 2014 in the area of a large number of municipalities of the Republic of Srpska, with emphasis on the territory of the municipalities of Banja Luka, Čelinac, Kotor Varoš, Gradiška, Srbac, Prnjavor and Derventa. Assessment of the occurrence and distribution of Jerusalem artichoke were taken along the roads in the urban areas, between the settlements in ruderal and less arable land, and on the edges of farmland, on arable land, along the river Sava and Vrbanja, as well as along the fish pond, in ditches and on the edges of forests. Quantitative distribution, study species density measure was assessed based on Blanque Braun (1964) method. During fieldwork Helianthus stands were mapped and recorded with GPS, while polygons were digitized using GIS software. All stands have been assigned to size classes (0-10m^2; 10-100m^2; 100-1000m^2; >1000).

Species habitat requirements

H. tuberosus prefers certain habitat types (Fehér and Končeková, 2001). It is best adapted to rich, moist soil that can be found along roadways, in wasteland areas and gardens (Wyse *et al*., 1986). According to Hartmann *et al*. (1995) *H. tuberosus* is completely naturalized on moist, nutrient-rich, sandy or loamy soils, especially along rivers. The most substantial site of occurrence includes riparian nitrophilic vegetation along watercourses, but also a variety of anthropogenic sites. In the landscape, it spreads very aggressively and settles more and more areas. It is contributed not only by spreading through generative diaspores, but also by vegetative propagation of broken-off parts of corms washed away by water to new sites along the watercourse (Cvachová *et al*., 2002).

Results and discussion

A wide-spread distribution of invasive weed species *H. tuberosus* was observed in the number of municipality of Republic of Srpska, specialy near the water sources in Banja Luka, Prnjavor, Vrbanja, Čelinac, Kotor Varoš and Derventa but as well between the above mentioned municipalities along the roads in populated areas, between settlements in ruderal and less arable land, on the edges of farmland and arable land, along the river Sava and Vrbanja, as well as along the fish pond, in ditches or even in the urban aerias (photo 2-7).

Photo 2. *H. tuberosus* between Banja Luka and Vrbanja

Photo 3. *H. tuberosus* in urban area of Derventa

Photo 4. *H. tuberosus* along the fish pond

Photo 5. *H. tuberosus* in corn crop

Photo 6. *H. tuberosus* on river bank of Vrbanja in Čelinac

Photo 7. *H. tuberosus* on the edges of farmland near Banja Luka

Regarding to other regions of Europe, *H. tuberosus* behaves as an invasive weed species in the studied region. Frequent expansion of the species into the surrounding sites inner town, near agricultural lands and the cultivated field is also noticed, beside the expansion on the river bank of Vrbanja and Sava. The abundance of Jerusalem artichoke points to a high density of individuals e.g. up to 70/m^2 along the fish pond between Prnjavor and Derventa, as well on river bank of Vrbanja and Sava (up to 30-40/m^2). *Helianthus tuberosus* can invide a resident plant communities and cause decrase in the number of native species or almost completely eliminate them from patches. As a result of the developing relationship of an invading plant a secondary, repeating combination of species is formed in which alien species play the main role (Falinski, 1998).

Only in the north western part of RS was recorded 40 Jerusalem artichoke stand. A half of polygons less than 100 m^2 were recorded in urban parts of north western area of Republic of Srpska, 15 polygons (100-1000 m^2) were recorded betwen Prnjavor and Derventa, Banja Luka and Čelinac, as well as five polygons (1000-10.000 m^2) (Photo 8_{1-34}).


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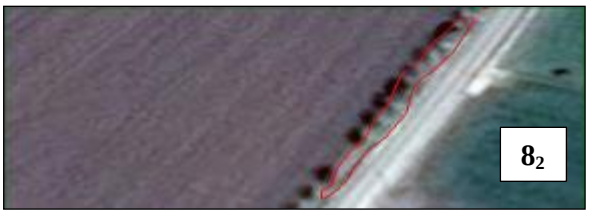
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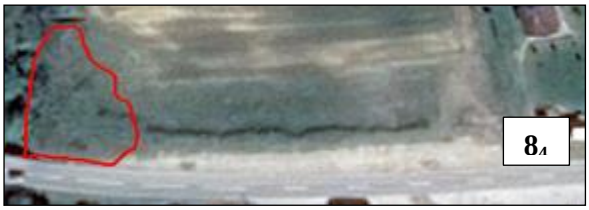
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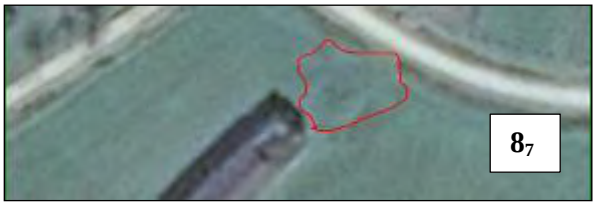
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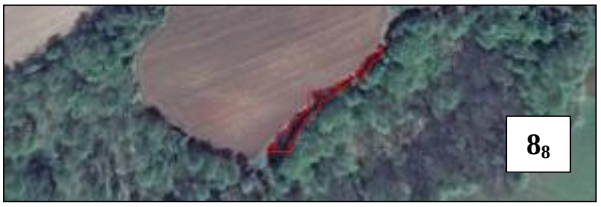
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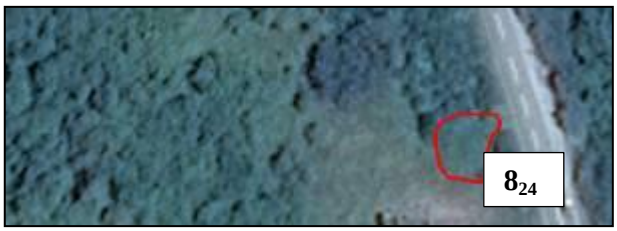
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Photo $8_{1\text{-}34}$. 34 Helinathus stand in the north western part of Republic of Srpska: polygons less than 100 m^2 in urban parts, areas between Prnjavor and Derventa, Banja Luka and Čelinac polygons less than 1000 m^2 and from 1000-10.000 m^2

Conclusion

Considering already conolized area by Jerusalem artichokea, number of finding stands in the studied region, high density of individuals/m^2, as well as recorded flooding waves in RS ponit out to certain further spreading of *H. tuberosus* as an invasive weed species. The most sever areas are in the river banks of Vrbanja and Sava, as well as areas along the fish pond between Prnjavor and Derventa.

Regarding to the current research in the north western part of Republic of Srpska jerulasem artichikea is in significant expansion, resulting in negative ecological, economic and social impact. Regarding to those research priorization process for mentonied area on local level is of a great importance. Under the given circumstances, undertaking comprehensive studies on the species characteristics and distribution potential on one hand and developing eradication and control methods, on the other are highly recommended.

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Original scientific paper
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CONTAMINATION OF SOIL AND PLANT MATERIAL IN FLOODED AREAS IN THE LOWER COURSE OF THE RIVER SPREČA (NORTHEASTERN BOSNIA AND HERZEGOVINA)

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Abstract

Floods in Spreča river basin (northeastern Bosnia and Herzegovina) carry all the negative elements that are naturally occurring and through anthropogenic activities.
The level of contamination in the soil above the threshold increases the risk of entering the food chain and thus for human health.
Particularly at risk is the quality of agricultural crops that are exposed to adverse effects, and there is accumulation of certain toxins in plants.
The aim is to study the contamination of soil and plant material caused by floods in the lower course of the river Spreča in the area of municipalities Lukavac and Gračanica.
Three profiles were opened on the three different soil types in those municipalities, and soil samples in disturbed and undisturbed condition were taken. General chemical and physical properties of the soil were analyzed in details and the level of the soil contamination with heavy metals and organic pollutants (PAH's) was determined. We also analyzed samples of plant material from cultures that were cultivated at the present microlocalities.
Based on determined state of soil and plants contamination, recommendations were given for the introduction of a permanent monitoring system for agricultural soils and plants in this area.
Keywords: *floods; contamination; heavy metals; PAHs;*

Introduction

The Government of Federation of Bosnia and Herzegovina (FBiH) adopted the conclusion in which Federal Institute of Agropedology is entrusted to conduct detailed research in the lower course of the river Spreča and to establish a three-year monitoring of the state of pollution of soil and plant material with heavy metals and organic pollutants (PAHs). The main task of the research is to determine the level of contamination of agricultural land and plant material (maize) with organic pollutants (PAHs) and heavy metals, such as: lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), chromium (Cr), arsenic (As), cobalt (Co), mercury (Hg) and copper (Cu). Limit values for heavy metals and organic pollutants were determined in accordance with the Law on Agricultural Land ("Official Gazette of BiH" No. 52/09), the Regulation on determining the permitable amount of harmful and hazardous substances in soil and methods of their usage ("Official Gazette of the Federation of Bosnia and Herzegovina "No. 72/09). For the plant material professional literature was used.
The aim of the research is to determine the level of contamination by heavy metals and PAH's in the flooded agricultural lands in the downstream river Spreča and their distribution in corn (root, stem, fruit). It is very important to be able to evaluate the possibilities of agricultural production and the safety of maize in the researched areas near flooded region.

Materials and methods

In the area of the lower course of the river Spreča we have examined the following parameters: soil texture by the International pipettes "B" method,

acidity of the soil in H_2O and 1M KCl (on pH meter Eutech PC2700) by method BAS ISO 10390:2009, dichromatic determination of humus (on colorimeter Hach Lange LICO 500), gas-volumetric determination of carbonate (on Scheibler's calcimeter) by method BAS ISO 10693:2000, heavy metals in extracts of soil with aquaregia (on atomic spectrometer AAnalyst 400 Perkin-Elmer) by methods BAS ISO 11466:2000 and BAS ISO ISO 11047:2000), heavy metals in plant material by wet digestion with HNO_3-$HClO_4$ mixture, direct determination of Mercury by Thermal Decomposition and Amalgamation (on SMS 100 Mercury Analyzer), PAHs determination by method BAS ISO 18287:2008 (on GC-FID).

Results and discussion

In the area of the lower course of the river Spreča, downstream of Lake Modrac, 3 pedological profiles on different soil types were opened. **Profile 1** was opened in Dobošnica, 35 m away from the Spreča river bed. The plot is sown with maize crops. This locality is represented with marsh *Gley soil - Eugley*. This land belongs to the class of hydromorphic department of gley soil. This land is characterized by groundwater which is mostly below 80 cm. Groundwater constantly fills the lower part of the profile, creating anaerobic conditions. The upper part of the profile is saturated with water only during one part of the year, and there is alternation between reduction and oxidation processes. (Resulović H.,Čustović H. i Čengić I. 2008). A total of 6 samples were taken in undisturbed condition, 2 samples in a disturbed condition and 1 sample of plant material – maize.

Soil profile No.1 (C.P.Dobošnica II)

Table 1. Average values of physical properties of the soil

Depth in (cm)	Bulk density (g/cm^3)	Volume density (g/cm^3)	Pore volume in (%)	The absolute capacity in (vol. %) for		K (cm/sec)	Designation of water permeability
				water	air		
0-14	2.56	0.89	64.76	52.09	12.67	0.44485	Very intensely permeable
14-39	2.57	1.05	59.06	45.06	14.00	0.04969	Very intensely permeable

The true specific density is very uniform in both layers. Bulk density was slightly lower in the first layer then in the second one. The air capacity is good for arable cultures (wheat and oats). Water capacity is in the first layer large, and in the second one slightly smaller. According to Gračanin, the first layer is determined as highly porous. The total porosity of the second layer is slightly lower. Permeability of the soil indicates that it is a very intensely permeable soil in both layers.

Table 2. Textural composition of the soil (texture mark by Ehwald)

Depth in (cm)	The percentage content of soil particles with a diameter in mm				Texture mark by Ehwald
	Coarse sand 2 – 0.2	Fine sand 0.2 – 0.02	Powder 0.02-0.002	Clay < 0.002	
0-14	1.70	39.00	43.80	15.50	**Loam**
14-39	1.47	39.23	43.70	15.60	**Loam**

The soil in both layers of this profile, according to the texture composition, belongs to the category of Loam. This profile is represented with coarse sand at smallest, and powder at the most.

Table 3. The chemical properties of the soil profile

Depth in (cm)	pH value in		The content of humus (in %)	The content of $CaCO_3$ (in %)
	H_2O	KCl		
0-14	8.53	8.01	3.74	32.15
14-39	8.72	8.02	3.37	34.13

Chemical analysis of soil indicates that this soil is highly alkaline, with pH value in H_2O from 8.53 to 8.72, and in 1 M KCl from 8.01 to 8.02. The humus content is medium. The content of calcium carbonate (CaCO3) is very high, so it is a very calcareous soil.

Table 4. The content of heavy metals and PAHs in soil and maize

Elements in soil (mg/kg of soil)	Depth of profile in (cm)		Parts of the plant (Corn)		
	0-14	14-39			
	Texture mark by Ehwald				
	Loam	Loam	Root	Stem	Fruit
Lead (Pb)	43.80	40.83	2.36	0.21	0.06
Cadmium (Cd)	**1.77**	**1.80**	0.90	0.07	0.02
Zinc (Zn)	71.80	70.83	19.25	11.75	11.87
Cobalt (Co)	28.77	28.27	5.90	0.26	0.04
Copper (Cu)	44.97	44.60	15.50	5.70	2.24
Chromium (Cr)	50.33	51.87	**42.00**	0.80	0.69
Nickel (Ni)	**340.67**	**321.67**	**180.20**	0.16	0.02
Mercury (Hg)	**2.443**	**2.715**	0.088	0.006	0.005
Arsenic (As)	11.22	12.28	1.50	n.d.	n.d.
Content - organic pollutants PAHs (mg/kg)					
PAHs	**4.265**	**4.837**	**1.747**	**1.34**	**1.481**

The contents of lead, zinc, cobalt,chromium, copper, and arsenic are below the limit values for that soil type -Loam. There is an elevated level of cadmium, in both layers, above the limit value of 1,00 mg/kg. Elevated level of nickel, is also present in both layers, and it is above the limit value for clay soil of 45,00 mg/kg. The limit value for mercury in clay soil is 1,00 mg/kg, and its content is elevated in both layers. The content of total PAHs, in both layers, is above the permited limit value of 2,00 mg/kg. According to the available literature (Kloke et al, 1984.), the presence of heavy metals in maize samples has been established. Red color represents content, critical for planth growth.

Profile 2 was opened in Donja Lohinja, 30 m away from the Spreča river bed. The plot is located in K.O. Donja Lohinja - Pribava, and k.č. is 2897. This plot is represented with *Alluvial soil - Fluvisol*. This type of soil belongs to hydromorphic department. Formed in the valleys of watercourses, it represents recent river alluviums applied in layers. Build of a profile is Ah - I - II-III. There were taken 12 samples in undisturbed condition, 4 samples in the disordered condition and 1

Soil profile No.2 (C.P.Donja Lohinja)

sample of plant material - maize crops.

Table 5. Average values of physical properties of the soil

Depth in (cm)	Bulk density (g/cm^3)	Volume density (g/cm^3)	Pore volume in (%)	The absolute capacity in (vol. %) for		K (cm/sec)	Designation of water permeability
				water	air		
0-26	2.95	1.32	54.91	40.47	14.43	0.39211	Very intensely permeable
26-66	2.67	1.62	39.36	28.69	10.67	0.00071	Medium permeable
66-110	2.76	1.43	48.18	36.01	12.17	0.01661	Very permeable
110-160	2.74	1.49	45.58	36.15	9.43	0.01478	Very permeable

The true specific density is greatest in the first layer of the profile, and lowest in the second. Bulk density is the lowest in the first, largest in the second,and in deeper layers of the profile almost evenly distributed. Water capacity is quite high in the first layer, and decreases in the following layers. According to Gračanin, the first layer is porous, whereas the others have a small porosity and decreases the penetration of the root system in the deeper layers. Permeability of the soil indicates that it is a very intensely permeable soil in the first layer, very permeable in the third and fourth, while in the second layer is medium.

Table 6. Textural composition of the soil (texture mark by Ehwald)

Depth in (cm)	The percentage content of soil particles with a diameter in mm				Texture mark by Ehwald
	Coarse sand 2 – 0.2	Fine sand 0.2 – 0.02	Powder 0.02-0.002	Clay < 0.002	
0-26	7.93	54.67	24.70	12.70	**Sandy Loam**
26-66	5.83	69.27	15.30	9.60	**Loamy sand soil**
66-110	10.12	73.08	9.70	7.10	**Sandy soil**
110-160	3.15	69.95	16.20	10.70	**Loamy sand soil**

In results shown above, least represented are the particles of coarse sand and clay, whereas mostly presented are fine sand particles.

Table 7. The chemical properties of the soil profile

Depth in (cm)	pH value in		The content of humus in (%)	The content of $CaCO_3$ in (%)
	H_2O	KCl		
0-26	8.47	7.63	1.95	4.34
26-66	8.43	7.08	0.56	0.16
66-110	8.44	7.61	0.54	1.01
110-160	8.19	7.49	0.46	0.93

The high pH values of water, in all layers, indicates very alkaline soil. The humus content is weak in all layers. The content of calcium carbonate (CaCO3) is low in all layers, and the land is poorly carbonated. The exception is the first layer, which is due to the value of 4.34% CaCO3, determined as medium calcareous soil.

Table 8. The content of heavy metals and PAHs in soil and maize

Elements in soil (mg/kg of soil)	Depth of profile in (cm)				Parts of the plant (Corn)		
	Tekxture mark by Ehwald						
	0-26	26-66	66-110	110-160	Root	Stem	Fruit
	Sandy Loam	Loamy sand soil	Sandy soil	Loamy sand soil			
Lead (Pb)	19.07	14.20	10.17	13.50	2.15	0.27	0.08
Cadmium (Cd)	0.97	**0.70**	**0.63**	**0.57**	1.00	0.08	0.04
Zinc (Zn)	42.73	35.17	30.07	34.33	18.40	8.54	10.85
Cobalt (Co)	30.40	28.80	23.57	26.00	**10.90**	0.12	0.24
Copper (Cu)	23.17	20.70	15.80	19.23	14.05	8.14	2.20
Chromium (Cr)	45.83	**68.17**	**60.77**	**60.73**	**33.35**	0.46	0.30
Nickel (Ni)	**498.33**	**467.00**	**352.33**	**429.33**	**245.70**	0.13	0.18
Mercury (Hg)	0.595	0.087	0.082	0.086	0.252	0.015	0.01
Arsenic (As)	4.828	4.225	3.525	4.15	2.20	n.d.	n.d.
Content - organic pollutants PAHs (mg/kg)							
PAHs	0.786	0.221	0.017	0.009	**0.294**	**0.212**	**0.413**

The contents of lead, zinc, cobalt, copper, mercury and arsenic is below the limit values. Elevated cadmium is present in the last three layers, ranging from 0.57 mg/kg to 0.70 mg/kg. Limit value for sandy soil is 0.50 mg/kg.
The content of chromium is increased in the last three layers, ranging from 60.73 mg/kg to 68.17 mg/kg and is above the limit value of 50.00 mg/kg for sandy soil. The increased nickel content is in all layers, ranging from 352.33 mg/kg to 498.33 mg/kg. Limit value of Ni for the sandy soil is 30.00 mg/kg.The total content of PAHs in all four layers of the soil is below threshold limit value of 2.00 mg/kg.
According to available literature (Kloke et al, 1984), the presence of heavy metals was categorized in samples of corn. The red color represents the metal content, that is critical for plant growth.

Profile 3 was opened in Stjepan Polje, 160 m away from the Spreča river bed. The plot is located in K.O. Gračanica, k.č. 5208. The sowing crop on the land is maize. *Eutric brown* soil is represented on alluvial deposits. This type of soil belongs to the class of automorphic department of cambic soils. Build of a profile is Ah - BV - IC. We had taken 12 samples in undisturbed condition, 4 samples in the disordered state and 1 sample of plant material - maize crops.

Soil profile No. 3 (C.P.Stjepan Polje)

Table 9. Average values of physical properties of the soil

Depth in (cm)	Bulk density (g/cm^3)	Volume density (g/cm^3)	Pore volume in (%)	The absolute capacity in (vol. %) for		K (cm/sec)	Designation of water permeability
				water	air		
0-18	2.68	1.25	53.11	39.44	13.67	0.09267	Very intensely permeable
18-38	2.54	1.54	39.25	37.25	2.00	0.00000	Watertight
38-76	2.64	1.28	51.50	33.90	17.60	0.01153	Very permeable
76-125	2.65	1.35	49.00	39.83	9.17	0.00328	Very permeable

The true specific density is the same in all the layers, except for slightly lower value in the second layer. Bulk density is the lowest in the first and the largest in the second layer. The air capacity is good in the first and third layer, while the value in second layer is very small. Water capacity is in all layers quite uniform and this ground can store medium amounts of water. According to Gračanin, soil is porous in the first, third and fourth layer, while the second layer is watertight. Water permeability of soil indicates that it is a very permeable soil except the second layer, where sole is created. This is impermeable layer.

Table 10. Textural composition of the soil (texture mark by Ehwald)

Depth in (cm)	The percentage content of soil particles with a diameter in mm				Texture mark by Ehwald
	Coarse sand 2 – 0.2	Fine sand 0.2 – 0.02	Powder 0.02-0.002	Clay < 0.002	
0-18	1.79	40.52	34.20	23.50	**Loam**
18-38	0.63	35.97	38.80	24.60	**Loam**
38-76	0.46	35.45	38.20	25.90	**Loam**
76-125	0.30	29.10	40.30	30.30	**Loamy clays**

At least represented are the particles of coarse sand. The values of fine sand particles decreases with depth. According to the texture composition, soil is characterized as Loam.

Table 11. The chemical properties of the soil profile

Depth in (cm)	pH value in		The content of humus in (%)	The content of $CaCO_3$ in (%)
	H_2O	KCl		
0-18	7.52	6.72	2.55	0.21
18-38	7.18	6.32	1.79	0.18
38-76	7.46	6.48	1.30	0.16
76-125	7.17	6.36	1.18	0.09

The values of pH in H_2O range from 7,17 to 7,52, respectively in 1M KCl from 6,32 to 6,74, and they indicate a neutral to slightly alkaline soil. The humus content is medium in the first layer, and its value decreases with depth. The content of calcium carbonate (CaCO3) is low, so it is a poor calcareous soil.

Table 12. The content of heavy metals and PAHs in soil and maize

Elements in soil (mg/kg of soil)	Depth of profile in (cm)				Parts of the plant (Corn)		
	Texture mark by Ehwald						
	0-18	18-38	38-76	76-125	Root	Stem	Fruit
	Loam	Loam	Loam	Loamy clays			
Lead (Pb)	22.27	20.23	21.00	23.47	1.18	0.30	0.11
Cadmium (Cd)	**1.03**	0.90	0.83	0.93	0.80	0.02	0.01
Zinc (Zn)	52.83	51.87	52.63	54.57	15.50	29.30	14.10
Cobalt (Co)	35.37	36.80	36.83	36.33	**3.40**	0.44	0.19
Copper (Cu)	32.67	35.17	36.37	40.20	14.00	6.40	2.05
Chromium (Cr)	**84.27**	74.23	73.53	74.47	**33.35**	0.46	0.30
Nickel (Ni)	**475.00**	**547.33**	**542.00**	**539.33**	**142.90**	0.15	0.11
Mercury (Hg)	0.141	0.113	0.121	0.140	0.04	0.01	0.01
Arsenic (As)	6.493	6.742	6.813	5.803	0.60	n.d.	n.d.
Content - organic pollutants PAHs (mg/kg)							
PAHs	0.400	0.049	0.002	0.001	**0.275**	**0.267**	**0.23**

The content of lead, zinc, cobalt, copper, mercury and arsenic is below the treshold value. Cadmium content of 1,03 mg/kg in the first layer is slightly above the treshold for a loam soil (1,00 mg/kg). The content of chromium in the first layer is above the limit value of 80,00 mg/kg for loam soil.The increased nickel content is present in all layers. Limit value of Ni for loam soil is 45,00 mg/kg. The total content of PAHs in all layers is below the treshold limit value of 2,00 mg/kg. According to available literature (Kloke et al, 1984), the presence of heavy metals is categorized in samples of corn. Yellow color represents content that exceeds the normal value, and red the content critical for plant growth.

Conclusions

Within conducted monitoring of land in the downstream river Spreča, three soil profiles on different soil types were processed. Profiles 1 and 2 are very alkaline, while the profile 3 is neutral. In all profiles, the soil has medium to low humus content. Very calcareous soil is represented in profile 1, while low carbonated is in profiles 2 and 3. Organic and inorganic pollutants were examined througout soil depths and in plant material. The profile 1 is recorded with increased content of cadmium, mercury and nickel, and the content of PAHs. In plant material, critical values of chromium and nickel at the root of maize and PAHs in all plant organs were detected. In the profile 2 there was an increased content of cadmium, chromium and nickel. PAHs are below the limit value. At the root of maize critical values of cobalt, chromium and nickel, and PAHs in all plant organs were detected. In the profile 3 we found an increased content of cadmium, chromium and nickel. PAHs are below the limit values. At the root of maize increased values of cobalt, a critical value of chromium and nickel, and PAHs in all plant organs were detected. In the stem and fruit of the maize, in all three soil profiles, the tested heavy metals values are within permissible limits, according to the available literature (Kloke et al, 1984). Elevated levels of cadmium, chromium, nickel and PAHs in this area are most likely caused by the anthropological influence, while for nickel it can be attributed to the lithological origin.

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THE RELATIONSHIP BETWEEN ANALYZED PHYSICAL, WATER AND CHEMICAL CHARACTERISTICS OF DIFFERENT AGRICULTURAL SOIL TYPES IN TUZLA CANTON

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Abstract

Canton Tuzla with a total area of 2,650 km^2 (265,000 ha), of which 68.37% (149,966.7 ha) is agricultural land, represents a very important basis for long-term development of agriculture and food industry in Bosnia and Herzegovina. Despite its importance however, the physical and chemical properties of the most important agricultural soils in this area make them less suitable for intensive agricultural production. A better understanding of soil physical, chemical and water properties of the most important agricultural soils in Tuzla Canton and analysis of their relationships, which is the main objective of this work, was done through analysis of: (i) chemical and physical properties of agricultural soils in this area; (ii) soil water properties through process of soil water retention and conductivity; (iii) correlation between the most important properties of selected agricultural soil types. Used research methods in order to meet the set goals were field work, laboratory work and data processing. Due to the obtained high value of the correlation coefficient between the analyzed physical, chemical and water characteristics for three types of agricultural soils in Tuzla Canton, one can say that usage of physical and chemical characteristics for the assessment of water characteristics in this area is justified. From the aspect of physical characteristics, this especially refers to content of clay, silt and sand, with which volumetric water content achieved strong or very strong correlations, such as correlation between clay content and plant water capacity (0.893 – 0.986).

Keywords: *Tuzla canton, soil characteristics, agriculture, correlation coefficient*

Introduction

Canton Tuzla with a total area of 2,650 km^2 (265,000 ha), of which 68.37% (149,966.7 ha) is agricultural land, represents a very important basis for long-term development of agriculture and food industry in Bosnia and Herzegovina. It is characterized, in its properties and quality, with very uneven land fund with dominant IVa, IVb, V and VI soil quality group (118,096.5 hectares or 78.8%) (Resulović et al. 2000). Most agricultural soils within the area are medium to heavy textured with high clay content (Vlahinić et al. 1967, Mehmedbašić, 1974). This Canton is an area rich in water, but also an area with a wide range of water issues, especially in agriculture. Unfavorable soil water - air regime is primarily due to its poor soil water and physical properties (Resulović, 2000). The rational and sustainable management of water and soil water regime is among the most powerful means of increasing agricultural production, while reducing its oscillations. Such management is achieved primarily by comprehensive analysis of physical, water and chemical properties of the soil and then, based on them, construction of adequate hydro-ameliorative facilities, which include facilities for drainage, irrigation and conservation of land and water. The possibility of different soil types for adsorption and retention of water, especially readily available water (RAW) is of great importance for agriculture. It is widely known (Jamieson 1958, Vlahinić 1967, Peterson 1967, Saxton 2006) that the texture, structure and content of organic matter in the soil have direct influence on its water - air properties. Research done by Debnath et al. (2012), based on analysis of influence of different physical and chemical characteristics of soil on its water

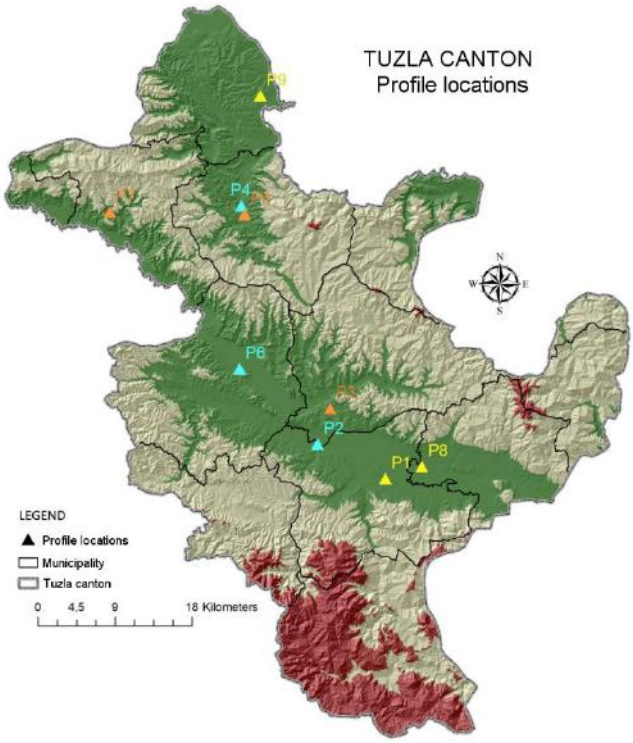
capacity, and taking into account the altitude, indicate that organic carbon, clay content, sand content and porosity directly affect the water capacity of the soil. A significant positive correlation was found between the water capacity of soil and organic carbon (r = 0.811 **) and clay content (r = 0.669 **), while a negative relationship was found with the bulk density (r = -0.315), sand content (r = -0.232), silt content (r = -0.252) and total porosity (r = -0.228). Also, higher pH values resulted in higher values of water capacity (r = 0.211). A better understanding of soil physical, chemical and water properties of the most important agricultural soils in Tuzla Canton and analysis of their relationships, which is the main objective of this work has been carried out through analysis of: (i) chemical and physical properties of agricultural soils in this area; (ii) soil water properties through process of soil water retention and conductivity; (iii) correlation between the most important properties of selected agricultural soil types. On the basis of above mentioned analyses and goals, possibility of indirect determination of curtain soil characteristics, especially soil water characteristics, based on other relatively easy to determine soil characteristics can be defined.

Materials and methods

Soil map of Bosnia and Herzegovina (Institute for Agropedology Sarajevo, 1990) was used to select three most important soils for agricultural production in the research area. On these three selected soils (Luvisol, Pseudogley and Fluvisol), nine soil profiles (up to 1 m deep) were opened. Undisturbed and disturbed soil samples were taken from each soil profile and each horizon in three replicates, making it 81 samples in total. Analyses of soil physical, water and chemical characteristics were carried out in laboratories of Institute for Pedology, Agrochemistry and Melioration at the Faculty of Agriculture and Food-science and the Federal Institute for Agropedology, Sarajevo. The following analyzes had been carried out: Soil physical characteristics through analysis of soil texture (*international pipette B method*) and bulk density (*gravimetric method in Kopecky cylinders*); Soil water characteristics through soil water retention (Θv) at a given matric potentials using a pressure-membrane extraction apparatus and Richards method (1941). Following matric potentials (Ψ) were used: -0.33 bar (pF 2.54: field water capacity), -6.25 bar (pF 3.8: lento-capillarity point) and -15.5 bar (pF 4.2: permanent wilting point). Totally (TAW) and readily (RAW) available water capacity of soil was calculated from following relation between filled water capacity (FWC), lentocapillary point (LCP) and permanent wilting point (PWP): TAW = FWC – PWP; RAW = FWC – LCP. Saturated hydraulic conductivity (K_{SAT}), was analyzed using undisturbed soil samples in Kopecky cylinders. K_{SAT} was measured in laboratory permeameter apparatus and calculated on basis of Darcy law. Analyzed soil chemical characteristics were active (H_2O) and substitution (KCl) pH reaction (electrometrically in suspension 1:2.5 - pH meter) and content of humus, using colorimetric method (spectrophotometry). Data processing was performed using correlation analysis in MS Excel. Strength of correlation is defined by the Roemer-Orphal scale.

Results and discussion

After the insight into soil map of BiH (1:50.000) and reviewing the situation in terms of spatial distribution and the importance for agricultural production, three soil types in the area of Tuzla canton were selected for this research: Luvisol, Pseudogley and Fluvisol. These are soils that are often used in agricultural production, occupying significant areas, mostly in the lowland part of the Canton (intensive agriculture favorable relief forms), and require a certain degree of melioration improvement. Figure 1 shows soil profiles and sampling locations in Tuzla canton. Profiles P3, P5 and P7 represent luvisol; Profiles P1, P8 and P3 pseudogley; and profiles P2, P4 and P6 fluvisol. After taking soil samples from the field and the laboratory analysis, the following average soil physical characteristics (Table 1) for these three soil types are determined.



All three soils in general are heavier textured, with significant clay content (28.21 - 59.55 %) which increases with depth. Fluvisol has a slightly higher sand content and the presence of skeleton compared to the other two soil types. Bulk density is within the limits of normal agricultural production. However, higher values in the second horizon (1.34 to 1.41 g/cm^3) in all the three soils, and a high content of clay (29.82 - 35.27%) indicate near surface presence of impermeable soil layer.

The average value of the volumetric water content (%) at different soil water capacity levels or matrix potentials (Ψ), as well as hydraulic conductivity (cm/h) for the three analyzed soil are shown in Table 2.

Figure 1: Soil profiles and sampling locations

Table 1: Soil physical characteristics

Soil type	Horizon	Depth (cm)	Skeleton (%)	Texture				Bulk density (g/cm^3)
				Send (%)	Silt (%)	Clay (%)	Class	
Luvisol	Ap	0 - 20	0.13	27.45	41.55	31.00	Clay loam	1.22
	E	20 - 45	0.97	25.71	39.02	35.27	Clay loam	1.36
	Bt	45 - 100	0.18	15.66	25.79	59.55	Clay	1.25
Pseudog.	Ap	0 - 23	0.21	25.74	46.05	28.21	Clay loam	1.15
	Sw	23 - 55		24.73	40.81	34.46	Clay loam	1.34
	Sd	55 - 100		20.95	36.40	42.64	Clay	1.41
Fluvisol	Ap	0 - 20	3.18	43.27	29.92	29.28	Clay loam	1.34
	I	20 - 60	6.86	45.20	26.62	29.82	Clay loam	1.41
	II	60 - 100	5.95	41.31	28.57	31.39	Clay loam	1.43

Table 2: Soil water characteristics

Soil type	Horizon	Depth (cm)	MWC	FWC	LCP	PWP	TAW	RAW	K_{SAT} (cm/h)
			-	2.54 (-0,33)	3.8 (-6,2)	4.2 (-15,5)	-	-	
Luvisol	Ap	0 - 20	54.26	43.68	21.30	15.89	27.80	22.38	5.65
	E	20 - 45	51.26	44.91	23.85	17.65	27.26	21.06	0.22
	Bt	45 - 100	55.11	51.78	32.02	28.09	23.68	19.76	3.11
Pseudogley	Ap	0 - 23	55.37	44.64	19.75	12.25	32.39	24.89	10.06
	Sw	23 - 55	50.43	47.73	22.67	15.25	32.49	25.07	2.87
	Sd	55 - 100	48.36	44.73	26.24	19.55	25.18	18.49	0.65
Fluvisol	Ap	0 - 20	50.58	43.08	24.61	15.42	27.67	18.47	30.25
	I	20 - 60	47.35	43.53	24.80	16.92	26.60	18.72	24.52
	II	60 - 100	46.56	39.95	24.81	18.21	21.75	15.15	18.14

MWC – maximum water capacity, FWC – field water capacity, LCP - lentocapillary point, PWP – permanent wilting point, TAW – totally available water, RAW – readily available water, KSAT – saturated hydraulic conductivity

The highest average value of a volumetric water content (VWC) at MWC is in the surface horizon (Ap) of Pseudogley, respectively 55.37%. Field water capacity (FWC) is the highest in the lowest (Bt) horizon of Luvisol (51.78%). In the case of VWC at the plant wilting point (PWP) the maximum value is in the Bt horizon of Luvisol (28.09), and the minimum at the surface horizon (Ap) of Pseudogley (12.25%). The amount of water at RAW capacity represents the most accessible form of soil water for plants and it is the main indicator of its retention characteristics. Pesudogley has the best retention properties from three analyzed soils. The Ap and Sw horizons of this soil can hold up to 25.07% of the volumetric water content. In terms of saturated hydraulic conductivity (K_{SAT}), the most permeable soil is Fluvisol, where average filtration coefficient in Ap horizon reaches 30 cm/h and gradually decreases with depth (down to 18.14 cm/h). Luvisol has the lowest values of K_{SAT}, especially in the second horizon (0.22 cm/h). Average basic chemical characteristics of three analyzed types of agricultural soils are shown in Table 3. Fluvisol proved to be the soil with best chemical characteristics. pH reaction of this soil ranges from slightly acid to neutral (6.16 – 6.91), and high humus content (3.31 %) slightly decreases with depth. Humus content is also high in Ap horizon of Pseudogley, strongly decreases with depth.

Table 3: Soil basic chemical characteristics

Soil type	Horizon	Depth (cm)	pH		Humus (%)
			H_2O	KCl	
Luvisol	Ap	0 - 20	6.09	5.13	2.87
	E	20 - 45	6.26	5.33	1.63
	Bt	45 - 100	5.94	5.07	1.14
Pseudogley	Ap	0 - 23	5.48	4.65	4.25
	Sw	23 - 55	5.02	4.21	1.64
	Sd	55 - 100	5.73	4.75	0.62
Fluvisol	Ap	0 - 20	6.96	6.16	3.31
	I	20 - 60	7.29	6.44	2.19
	II	60 - 100	7.69	6.91	1.52

On the basis of the results for all the analyzed soil characteristics (physical, chemical, and water), and based on all the individual measurements (27 for each soil type), the correlation analysis had been conducted for each type of soil in particular (Tables 4, 5 and 6). We will mainly focus on the impact of physical and chemical characteristics on the soil water properties.

In Luvisol (Table 4), higher clay content results in higher values of the LCP, PWP, FWC and MWC and lower values of TAW and RAW. According to the strength of correlation, clay

content has the following effects on water characteristics of Luvisol: LCP (r = 0.937) > PWP (r = 0.932) > FWC (r = 0.895) > TAW (r = -0.558) > MWC (r = 0.454) > RAW (r = -0.266) > K_{SAT} (r = -0.191). Bulk density has a strong negative relationship (r = -0.911) with the MWC, and the greatest impact (r = -0.406) on the hydraulic permeability compared to all other observed soil characteristics. Regarding soil chemical characteristics, pH does not have any significant effect, except to RAW with a middle correlation coefficient (r = -0.427). However, the humus content has a strong negative impact on the water content in the LCP (r = 0.613), FWC (r = -0.595) and PWP (r = -0.552).

In case of Pseudogley (Table 5), the higher clay content means higher values of LCP and PWP and lower values of MWC, TAW and RAW. The sand and silt content in this soil have a reverse effect. More of these particles means lower values of LCP and PWP and higher values of TAW and RAW. Of the three textural elements of soil, clay content has the greatest impact on water properties, in this order: PWP (r = 0.986) > LCP (r = 0.977) > TAW i RAW (r = -0.754) > MWC (r = -0.724) > K_{SAT} (r = -0.284) > FWC (r = 0.245). Bulk density also has a significant impact on the water properties of Pseudogley, behaving similarly as clay content.

Table 4: Correlation coefficient (r) between different soil characteristics in Luvisol

LUVISOL	*C*	*S*	*Sa*	*Bd*	*MWC*	*FWC*	*LCP*	*PWP*	*TAW*	*RAW*	*K_{SAT}*	*pH H*	*pH K*	*H*
Clay	1													
Silt	-0.979	1												
Sand	-0.965	0.890	1											
Bulk Density	-0.217	0.185	0.245	1										
MWC	0.454	-0.453	-0.426	-0.911										
FWC	0.895	-0.892	-0.843	-0.108	-0.911	1								
LCP	0.937	-0.931	-0.886	-0.050	-0.881	0.955	1							
PWP	0.932	-0.952	-0.846	-0.091	-0.812	0.919	0.970	1						
TAW	-0.525	0.582	0.418	0.011	0.203	-0.290	-0.501	-0.643	1					
RAW	-0.266	0.256	0.261	-0.182	0.023	0.014	-0.282	-0.299	0.755	1				
K_{SAT}	-0.191	0.116	0.277	-0.406	0.282	-0.198	-0.215	-0.131	-0.067	0.083	1			
pH H_2O	-0.468	0.431	0.485	0.441	0.152	-0.417	-0.274	-0.237	-0.236	-0.427	0.041	1		
pH KCl	-0.373	0.319	0.419	0.442	0.100	-0.342	-0.195	-0.125	-0.361	-0.452	0.044	0.978	1	
Humus	-0.725	0.715	0.693	-0.053	0.496	-0.595	-0.613	-0.552	0.184	0.143	0.256	0.655	0.585	1

C – Clay, S – Silt, Sa – Sand, Bd – Bulk density, MWC – maximum water capacity, FWC – field water capacity, LCP - lentocapillary point, PWP – permanent wilting point, TAW – totally available water, RAW – readily available water, KSAT – saturated hydraulic conductivity, pH H – pH in H2O, pH K – pH in KCl, H - Humus

Higher bulk density or greater soil compaction results in lower values of MWC, TAW, RAW and K_{SAT} and higher values of LCP and PWP. Regarding soil chemical characteristics, strong negative correlation between pH in H_2O and TAW (r = -0.597) and RAW (r = -0.566) can be noted. The humus content has a significant impact on the soil water properties and behaves similarly to silt, and inversely to the clay content. Humus, of all the analyzed characteristics, has the biggest positive impact on saturated hydraulic conductivity (r = 0.499), which means the higher humus content, the better soil permeability.

Table 5: Correlation coefficient (r) between different soil characteristics in Pseudogley

PSEUDOGLEY	*C*	*S*	*Sa*	*Bd*	*MWC*	*FWC*	*LCP*	*PWP*	*TAW*	*RAW*	*K_{SAT}*	*pH H*	*pH K*	*H*
Clay	1													
Silt	-0.921	1												
Sand	-0.415	0.027	1											
Bulk Density	0.702	-0.576	-0.457	1										
MWC	-0.724	0.708	0.203	-0.928	1									
FWC	0.245	-0.292	0.053	0.155	-0.207	1								
LCP	0.977	-0.874	-0.465	0.746	-0.732	0.298	1							
PWP	0.986	-0.868	-0.502	0.738	-0.711	0.223	0.991	1						
TAW	-0.754	0.615	0.497	-0.582	0.524	0.435	-0.724	-0.781	1					
RAW	-0.754	0.623	0.479	-0.598	0.549	0.424	-0.738	-0.783	0.995	1				
K_{SAT}	-0.284	0.236	0.179	-0.571	0.573	-0.316	-0.339	-0.313	0.087	0.098	1			
pH H_2O	0.529	-0.556	-0.061	0.023	-0.187	-0.229	0.425	0.487	-0.597	-0.566	0.143	1		
pH KCl	0.240	-0.255	-0.022	-0.332	0.156	-0.243	0.113	0.172	-0.315	-0.280	0.316	0.819	1	
Humus	-0.751	0.593	0.539	-0.891	0.747	-0.112	-0.762	-0.789	0.657	0.645	0.499	-0.056	0.282	1

C – Clay, S – Silt, Sa – Sand, Bd – Bulk density, MWC – maximum water capacity, FWC – field water capacity, LCP - lentocapillary point, PWP – permanent wilting point, TAW – totally available water, RAW – readily available water, KSAT – saturated hydraulic conductivity, pH H – pH in H2O, pH K – pH in KCl, H - Humus

In Fluvisol (Table 6), content of the silt particles have reversed effect compared to the other two soil types. In this soil, silt increases the value of the LCP (r = 0.936), FWC (r = 0.888) and PWP (r = 0.750), and reduces the value of RAW (r = -0.419). According to the strength of the correlation, clay content has the following effect on water properties of fluvisol: LCP (r = 0.991) > FWC (r = 0.907) > PWP (r = 0.893) > RAW (r = -0.535) > MWC (r = 0.493). Increased bulk density results in smaller values of VWC at all water capacities. Substitution (KCl) pH reaction has a strong negative relationship with the TAW (r = -0.726) and a very strong negative relationship with the FWC (r = -0.790). The content of humus in fluvisol, in relation to luvisol and pseudogley, except MWC (r = 0.700) and TAW (r = 0.493) also have a positive effect on values of FWC (r = 0.726), LCP (r = 0.596) and PWP (r = 0.345).

Table 5: Correlation coefficient (r) between different soil characteristics in Fluvisol

FLUVISOL	*C*	*S*	*Sa*	*Bd*	*MWC*	*FWC*	*LCP*	*PWP*	*TAW*	*RAW*	*K_{SAT}*	*pH H*	*pH K*	*H*
Clay	1													
Silt	0.934	1												
Sand	-0.979	-0.988	1											
Bulk Density	-0.510	-0.658	0.603	1										
MWC	0.493	0.619	-0.574	-0.947	1									
FWC	0.907	0.888	-0.911	-0.577	0.565	1								
LCP	0.991	0.936	-0.976	-0.540	0.513	0.944	1							
PWP	0.893	0.750	-0.826	-0.375	0.325	0.718	0.868	1						
TAW	0.001	0.168	-0.098	-0.260	0.312	0.359	0.085	-0.392	1					
RAW	-0.535	-0.419	0.477	0.071	-0.018	-0.143	-0.461	-0.674	0.713	1				
K_{SAT}	0.193	0.293	-0.256	-0.374	0.423	0.229	0.172	-0.082	0.334	0.009	1			
pH H_2O	-0.550	-0.607	0.592	0.550	-0.530	-0.766	-0.640	-0.249	-0.679	-0.141	-0.152	1		
pH KCl	-0.563	-0.619	0.604	0.530	-0.542	-0.790	-0.650	-0.238	-0.726	-0.177	-0.220	0.988	1	
Humus	0.515	0.662	-0.608	-0.748	0.700	0.726	0.596	0.348	0.493	0.165	-0.003	-0.790	-0.772	1

Conclusion

All three soils in general are heavier textured, with significant clay content (28.21 - 59.55 %) which increases with depth. Higher values of bulk density in the second horizons (1.34 to 1.41 g/cm^3) in all the three soils, and a large content of clay (29.82 - 35.27%) indicate near surface presence of impermeable soil layer. In average, Pseudogley has the biggest capacity for the plant readily available water (RAW) and good permeability of the surface layer. This soil compared to other analyzed soils showed the best soil water characteristics in terms of agricultural production. Although it has good retention properties, Luvisol has highly impermeable second layer, while Fluvisol has poor retention capabilities due to high hydraulic conductivity. Due to the obtained high value of the correlation coefficient between the

analyzed physical, chemical and water characteristics for three types of agricultural soil in Tuzla Canton, one can say that usage of physical and chemical characteristics for the assessment of water characteristics in this area is justified. From physical characteristics, this especially refers to content of clay, silt and sand, with which VWC achieved strong or very strong correlation. From the chemical characteristics aspect, content of humus has the greatest impact on water characteristics of these analyzed soils.

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ASSESSING THE HYDRAULIC SENSITIVITY OF PRESSURIZED IRRIGATION DELIVERY NETWORKS THROUGH THE MASSCOTE/MASSPRES RATIONAL

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Abstract

The present study aimed at reinforcing an existing methodological framework for Diagnostic Performance Assessment (DPA) of large-scale irrigation systems with an analytical procedure for evaluating the hydraulic response of pipe delivery networks to changes in flow conditions. This framework is named Mapping System and Service for Pressurized Irrigation Systems (MASSPRES) and was originally conceived within a joint effort between FAO and CIHEAM-IAM Bari for Irrigation System Modernization in the Near East and North Africa (NENA) Region. Sudden changes of flow rate and/or piezometric elevation at the inlet of irrigation distribution systems may impact the water delivery service to farmers at different turnouts or delivery points (hydrants), on the basis of the network's hydraulic response and sensitivity to those flow changes. The analytical procedure entailed the use of a hydraulic simulation model to generate flow scenarios and assess the hydraulic response of the pipe network to flow changes, and the resulting impacts at different hydrants. Two delivery performance indicators were used, namely the relative pressure deficit (RPD), and its likely expected value (EV), which were combined into a composite operational index (OI). The procedure was applied for validation to an existing pipe irrigation system of southern Italy in need of modernization. Different flow scenarios were simulated for the pipe network under study, followed by an evaluation of its response and impacts to delivery targets at hydrants level. Results showed that the proposed procedure and operational index could be useful to identify critical areas of irrigation distribution networks, where failures are likely to occur, and where the attention of operational staff should focus to ensure adequate and equitable water delivery services throughout the irrigation system.

Keywords: *irrigation delivery systems, diagnostic performance analysis, hydraulic response, irrigation system modernization.*

Introduction

Worldwide, the agricultural sector is by far the largest user of fresh water, as it accounts for 67% of the world's total fresh water abstraction and 86% of its consumption (Jacobs et al., 2008). However, a major part of the irrigated areas is served by large-scale distribution systems whose performances are often way below expectations (Renault et al., 2007). In this view, improvements are needed in water resources management, irrigated agriculture and asset management. In the Mediterranean countries the agricultural water demand increase over space and time. Blinda and Thivet (2006) report that agricultural water use currently accounts for 63% of the total water demand, thus it is needed to put the available fresh water reserves under increased pressure. The agricultural sector in those countries uses on average more than 70% of total water, with nearly 50% in the Northern Mediterranean countries, and 79% in the southern Mediterranean countries (Jacobs et al., 2008). In the recent years, several scientists (Burt, 1996; Burt and Style, 1999; Clemmens, 2006; Playan and Mateos, 2006) emphasized that managing irrigation systems and improving the performance of existing

irrigation schemes are very critical activities to enhance their productivity. In this context, current levels of performance must be systematically assessed and the most effective measures for improvements should be identified.

The present study aimed at testing the reliability of the MASSPRESS methodology in analyzing the operation of pressurized irrigation delivery networks for addressing modernization aspects. The study entailed hydraulic simulations on a poor-performing irrigation system located in southern Italy and managed by a local Water Users Organization (WUO), with a focus on analyzing the performance achievable at the hydrant level under different operational scenario, through the application of hydraulic performance indicators. In detail, the study included the following activities: (1) Modeling the capacity and sensitivity of the system; (2) Defining and validating a specific indicator to allow evaluating the hydraulic response of the pipe network to changes in flow conditions; (3) Evaluating how the delivery service at hydrants would be impacted at hydrant level in response to flow changes and to the network's hydraulic behavior.

Materials and Methods

Rationale of the proposed methodology

For pressurized irrigation distribution networks there is no specific definition or indicator of the hydraulic sensitivity, which is a relevant parameter for system design and modernization to evaluate the network's behavior in propagating/attenuating flow perturbations. The approach utilized within the present study entailed simulating and analyzing the performance achievable by the pipe distribution network in terms of delivery service at hydrants. The COPAM simulation model (FAO, 2008) was utilized in this study for the performance evaluation on the basis of the hydraulic behavior of the pipe distribution network. The network's hydraulic behavior and resulting impact on irrigation delivery service at hydrants can be investigated by means of hydraulic simulation models, and can be physically represented in terms of flow rates and pressure heads available to farmers for adequately operating their on-farm irrigation systems downstream of delivery hydrants. In detail, irrigation delivery performance at hydrants can be represented by the values of the relative pressure deficit (RPD) in a plan where the abscissas correspond to the hydrant numbering and the ordinates to the RPD value. This can provide a good understanding of the hydraulic behavior of each hydrant during operation, showing (1) How much pressure head is available at each hydrant under different flow configurations (2) How often the hydrant is unsatisfied with respect to minimal conditions for adequate operation of the on-farm irrigation system by farmers (3) How much variation of pressure head occurs at hydrants as a result of different flow configurations.

Operational index

An innovative approach was tested during the present study that is based on the combination of the hydraulic indicator of relative pressure deficit (RPD), used within the COPAM software package, and a statistical parameter; "the expected value" (EV) of the RPD. Specifically, the EV was integrated with the variation of the RPD values in one equation for the determination of a combined index named Operational Index (OI), which could provide meaningful and detailed information about the impact of flow changes on irrigation delivery service at hydrants. The expected value of a random variable is the weighted average of all possible values that this random variable can take. The weights used in computing this average correspond to the probabilities in case of a discrete random variable, or the densities in case of a random variable. From a theoretical standpoint, the expected value is the integral of the random variable with respect to the measure of its probability. Supposed that the random variable X can take value x_1 with probability p_1, value x_2 with probability p_2, and so on, up to value x_k with probability p_k, and then the expected value of the random variable X is defined as:

$$EV(X) = x_1 p_1 + x_2 p_2 + ... + x_k p_k \quad (1)$$

Since all probabilities p_i add up to 1 (p_1+p_2 + ... +p_k= 1), the expected value can be viewed as the weighted average, with p_i's being the weights

$$EV(X) = \frac{\sum_1^k x_i p_i}{\sum_1^k p_i} \quad (2)$$

Where: EV(X) is the expected value of a random variable With X_i being the value of the variable (in our case the RPD values); $p_{i\ is}$ the probability of occurrence of each value X_i

The use of EV for evaluating the effects of the network's hydraulic behavior at hydrant level thus allows computing the weighted average of the entire set of RPD values accounting for their probability of occurrence. However, the use of EV alone could not provide comprehensive and exhaustive information on the network performance and sensitivity, as more than one hydrant could have the same EV, thus not allowing a comparative evaluation of their sensitivity. In the same value of expected value which means that we can't understand which one is more sensitive comparing to others. As such, the EV must be combined with other indicators to allow determination of hydrants' sensitivity and get information about the most sensitive areas of the network as a result of external changes. Given that the RPD provides detailed information on the spatial variability of the hydrant pressure, and also the range of fluctuation of the pressure head occurring at the different hydrants, it is the most suitable indicator to be combined with the EV. The parameter resulting from the above-indicated combination is named Operational Index (Oi) and is obtained through equation 3 in such a way to indicate how changes in flow conditions affect the hydrants both in terms of available pressure head and range of its variation.

$$OI = (\frac{Adequacy - EV}{MaxEV}) * 10 + (\frac{\Delta RPD}{Max\Delta RPD}) \quad (3)$$

Where: OI: is the operational index (unit less); Adequacy is the zero-value line of the relative pressure deficit, corresponding to the minimum pressure head necessary for adequate on-farm irrigation (ex. Adequacy = 15 m); EV is the expected value of RPD (m); ΔRPD is the range of variation of the RPD value at each hydrant (m). The equation 3 produces values of the OI ranging in a scale between – 10 and + 10

The operational index ranges

As explained in the previous section, the OI provides information about the hydrant's RPD value with respect to the adequacy line; and it also allows knowing the relative distribution of RPD values according to their probability of occurrence. As such, it allows comparisons between hydrant's performance under the same flow conditions (where the hydrant's performance can vary as a result of the different configuration of hydrants in simultaneous operation), and when flow changes occur in the network.

A tentative set of reference standard values was identified to allow ranking and classifying hydrant's performance based on their sensitivity to changes in network's operating conditions. The ranking procedure is composed of the following elements: The maximum range of variation of the OI values set between -10 and +10.

An upper boundary calculated on the basis of the nominal operating pressure stood by the most commonly used pipes for on farm irrigation system downstream of the delivery hydrants. In case of plastic pipes with nominal operating pressure limit of 6 bars (~ 60 m), the upper boundary can be calculated as

$$OI = (\frac{15 - 60}{60}) * 10 = -7.5 \quad (4)$$

A range of "Highly Sensitive" is defined by OI values between -7 and -10

A lower boundary is set according to the minimum value of pressure head at hydrants that is compatible with the proper operation of farm irrigation networks (~ 20 m). The lower boundary is thus calculated as:

$$OI = (\frac{15-20}{20}) * 10 = -2.5 \qquad (5)$$

A field of “Slightly Sensitive” is defined by OI values between 0 and -2.5, whereas the hydrant will be classified as “Sensitive” if the value of OI results between -2.5 and -7.
The field of “Poorly performing” is when the pressure head available at hydrant (H) is lower than the minimum required for proper operation of farm irrigation system.

Table 1. Tentative values of OI for ranking hydrants in terms of sensitivity

	-10		-7		-2.5		0		10
Highly sensitive (HS)	⦶	+	⦶						
Sensitive (S)			⦶	+	⦶				
Slightly sensitive (SS)					⦶	+	⦶		
Poor-performing (PP)							⦶	+	

Description of the study area

The Sinistra Bradano irrigation scheme is located in southern Italy, in the western part of the province of Taranto and covers a total topographic area of 9,651 ha. The system is divided into 10 operational units called districts, each of them subdivided into sectors. The present study focused on the District 10, which is composed of three sub-areas that are served by three distribution sub-networks originating from as many different diversions along the last section of the main canal, namely the Diversion 7 (D7), 8-North (D8-N) and 8-South (D8-S). The various simulations and performance analysis were conducted on the sub-network 7.

Application of the proposed methodology to the selected irrigation districts

A number of simulations were conducted on the irrigation delivery sub-network 7 that entailed three main scenarios described hereafter: (1) a fixed upstream piezometric elevation at 60 m a.s.l. and different values of the available flow rate at the network inlet, varying from Q_{up1} = 220 l s^{-1},to Q_{up2} = 240 l s^{-1} and Q_{up3} = 260 l s^{-1}; (2) the upstream discharge is fixed at 240 l s^{-1} and different values of the upstream piezometric elevation, varying from Z_{up0} = 60 m, to Z_{up1} = 70 m, Z_{up2} = 75 m, and Z_{up3} = 80 m. (3) this scenario consists in evaluating the sensitivity at hydrants without changing the flow parameters at the network inlet. In this case, only the configuration of hydrants in simultaneous operation could change for the same value of flow rate and piezometric elevation at the network inlet. This scenario will provide information on the variability of delivery performance at hydrants within the network as a result of the changes in configuration of hydrants in operation. The three scenarios will result in different delivery performance at hydrants, which can be evaluated and ranked through the Operational Index. Values of the OI can be presented in a plot and show the effect on the hydrants resulting from changes in the flow parameters at the network inlet. The analysis of the above-defined scenarios allowed (i) Identification of “weak” areas within the distribution network, which require operational attention (ii) information about the delivery performance at hydrants when the flow conditions in the network deviate from a specific set point, i.e. changes in available flow rate or piezometric head at the network inlet; (iii) testing the applicability of the set of proposed reference values of the OI, for ranking and classifying hydrants on the basis of their sensitivity to flow changes.

Results and Discussion

Figure 1 show the results of simulations conducted on the first sector, compared by the first thirteen hydrants, showed identical three values of OI, these hydrants are not sensitive to the changes made, while the rest of hydrants showed different OI values as resulting from changes of upstream flow rate. So, passing from Q_{up1} to Q_{up2} and Q_{up3} a similar pattern was observed for hydrants having different OI values. Figure 2 shows that if the upstream piezometric elevation Z_{up} is progressively increase of 5m increments, the OI values will shift

upwards meaning that the network reacts to piezometric elevation increases with higher pressure heads at the delivery hydrants, as expected.

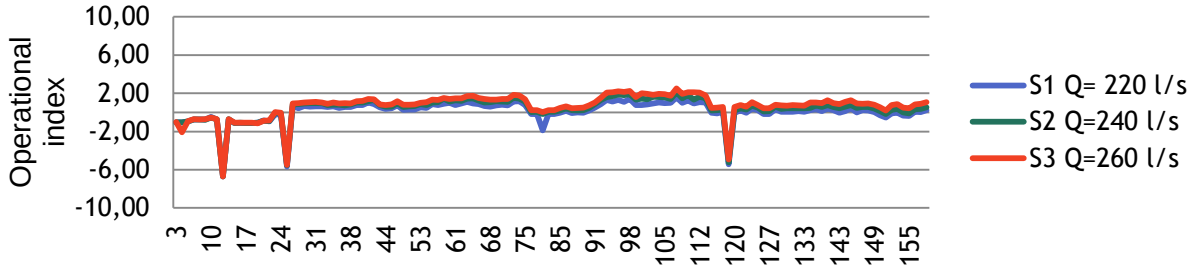

Figure.1.Values of OI resulting from the changes indicated in the first scenario (Z_{up} fixed and Variation of Q_{up})

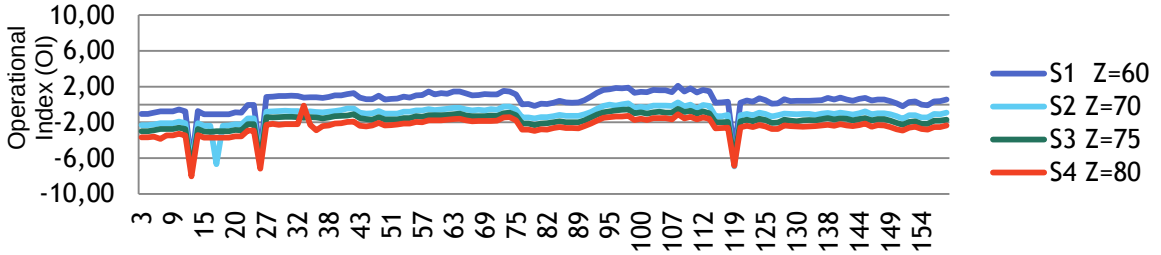

Figure.2. Patterns of the OI values that result from progressive increases of the upstream piezometric elevations (Q_{up} fixed and Variation of Z_{up})

Lamaddalena and Sagardoy (2000) report that at certain number of configurations with respect to the number of hydrants in the network, the system stabilizes and don’t react regarding to the changes made. Indeed, this was confirmed by this scenario where three sets of 500 configurations were generated, indicated in Figure 3. Thus, it can be noticed that three curves are identical, meaning that the network stabilizes and there is no changes with respect to the changes made.

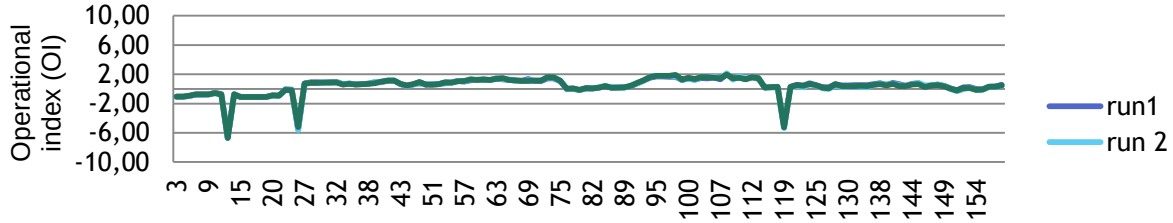

Figure.3. Values of the OI within the configurations for the third scenario (No changes in inputs)

Figure 4 shows the case of hydrants having the same EV but different RPD variation. The hydrants with number 74 and 98 have both EV of -0.14, a range of variation of the RPD Thus, the OI values allow ranking the hydrants in order of sensitivity as follows: Hydrant 98 > Hydrant 74. Figure 5 refers to the case of hydrants having different EV and different values of RPD variation. The hydrants numbered 24 and 127 were compared, that have EV of 0.9 and 0.84, RPD variations of 0.43 and 0.93, whereas the OI values are -1.55 and -0.22, respectively. Based on OI values, the hydrant 24 is considered more sensitive to the changes than the hydrant 127. Figure 6 presents the case of the hydrants with different EV and the same RPD variation. The hydrants numbered 23 and 76 are compared, with EV of 0.22 and

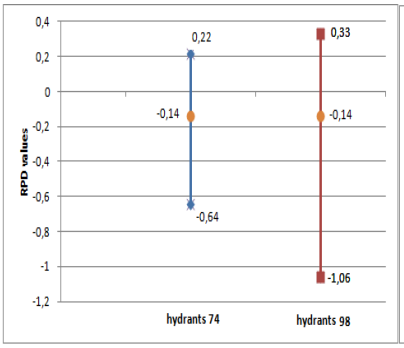

Figure.4. Case of hydrants having same EV and different ranges of RPD variations (Scenario1, Simulation 1)

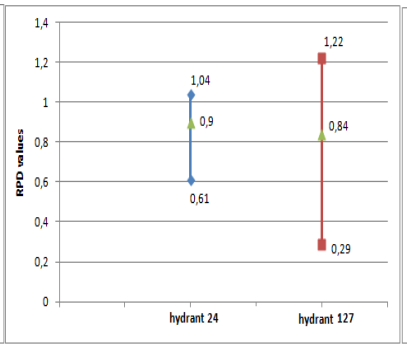

Figure.5.Case of hydrants having different EV and different ranges of RPD variations (Scenario2, Simulation1)

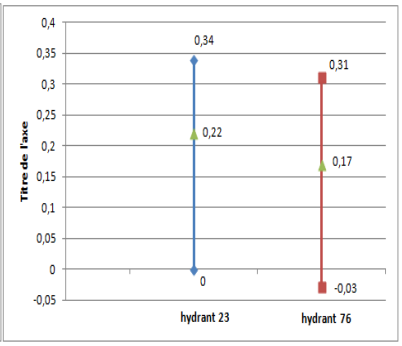

Figure.6. Case of hydrants having different EV and same ranges of RPD variations (Scenario3, Simulation 2)

0.17, RPD variation for both of 0.34, whereas the OI values are -0.13 and 0.01, respectively. Based on OI values, hydrant 76 is considered more sensitive to changes than the hydrant 23.

Conclusions

The operational index developed and applied in this study allowed to better understand the impact of flow changes on delivery performance at hydrants in the pressurized irrigation distribution network analyzed. In addition, it enabled to combine different performance indicators into one single index which includes (a) the probability of occurrence of different values of the RPD, through the statistical parameter of the expected value, (b) the range of fluctuation of the pressure head occurring at hydrants through the variation of the relative pressure deficit (RPD) and (c) the possibility to rank the hydrant performance based on the operational index. This composite index provides information about the relative position of the pressure head occurring at each hydrant with respect to the adequacy line (zero RPD), and indications on the hydrant's sensitivity to changes of flow conditions through the irrigation distribution network.

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LAND SUITABILITY EVALUATION FOR VEGETABLE CROPS IN PLOVDIV REGION, BULGARIA, USING GIS APPLICATION

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Abstract

Land suitability evaluation is a way of systematically collecting and analyzing different kind of information. It contains interdisciplinary information that involves integration of criteria from different branches of science. All gathering data analysed and converted into spatial database. Level information is arranged by classifying the main factors for vegetable growing, especially green beans one. The area of this research is Perushtitza Village, Plovdiv Region, Bulgaria. This is a report of multi-criteria land suitability evaluation, in which variety dimensions are incorporated into GIS technical tool. For ranking and important judgment of the criterias, pair-wise comparison using Analytical Hierarchy Process (AHP), developed by Saaty (1977), was carried out. The results are portrayed in the thematic map of the Perushtitza Village. The main objective of this study is to classify available agricultural land area of Perushtitza valley into different suitable classes for vegetable crop cultivation, using GIS application. The core of process is to create assessment map by calculating important indicators for sustainable vegetable cultivation.

Keywords: *vegetables, land suitability evolution, GIS, assessment, Analytical Hierarchy Process (AHP)*

Introduction

Horticulture is one of the most spread cultivation practice in Bulgaria. Its good natural and environmental factors make possible to increase yield over the years. Vegetable production systems in Plovdiv region is land form, farm size and intention of cultivation. Sustainable vegetable farming system is associated with good practices related to people cantered development, sustainable livelihood, agro-ecological practices, sustainable forestry system, community based natural resources management, participatory policy development, indigenous farming system, fair labour condition, good agricultural practises, equitable access to water and others (Baniya, 2008). Farming system corresponds closely with the land use planning. Physical land suitability evaluation is carried out with analysis of soil characteristics, land characteristic and climate characteristic. Sustainability is the ability of an agricultural system to meet evolving human needs without destroying and, if possible, by improving the natural resource base on which it depends (USAID, 1988). Land suitability evaluation can also be defined as the assessment or prediction of land quality for specific use. This process includes identification, selection and description of land use types relevant to the area under consideration; mapping and description of the different types of land that occur in the area and the assessment of the suitability of the different types of crops. In this research selected area is Plovdiv Region and especially Perushtica Village. This region is selected by its plane relief and suitable environmental parameters for vegetable growing.

This study presents the influence of physical land characteristics on green beans development. It includes deeply analyses on soil and relief parameters, needed for successfully crops growing.

Materials and methods

Initially used based information about green beans developing is soil maps, digital cadastral maps into appropriate coordinate system, climate characteristics, summarized parameters for established crops growing. The methodology is based on matching soil and relief characteristics, both named as Physical land characteristics and agronomical requirements of green beans. Using GIS tools for analysing the suitability classification will be assessed. Based on analysing tools and recommended crops parameters are made maps of land suitability accordingly to soil and relief parameters.

The organization of database is presented by Analytic Hierarchy Process (AHP), introduced by Saaty (1977). By making pair-wise comparisons at each level of the hierarchy, participants can develop relative weights to differentiate the importance of the criteria (Hossain et.al. 2007). Structure of assessment about criteria and sub-criteria is made by the alternatives, indicating the degree of suitability with respect to a criterion. Final thematic maps of every one of the studied parameters present level of land suitability for green beans development. By AHP method all parameters are compared using matrix for physical land criteria and sub-criteria about soil and relief.

The local priorities are multiplied by the weights of the respective criterion. The results are summed up to get the overall priority of each alternative. For each level in the hierarchy it is necessary to know whether the pair-wise comparison has been consistent in order to accept the results of the weighting. The Consistency Ratio (CR) is a measure of how much variation is allowed for reasonable results that is expected to be less than 10 per cent for the reasonable result. CR calculation is described as in following formula from the matrix Goal calculation, λmax value can be gained and later it is used to count Consistency Ratio (CR) and Wi which becomes the priority vector. The formula of Consistency Ratio (CR) got from the Consistency Index (CI) is as follows: $CI = (\lambda max - n) / (n - 1)$ and $CR = CI / RI$

Where: λmax: The maximum value; CI: Consistency Index; CR: Consistency Ratio ; RI : Random Index; n: The numbers of criteria or sub-criteria in each pairwise comparison matrix

Random Index (RI) says that the average of consistency of comparative matrix in pairs is 1-10, got from the experiment of *Oak Ridge National Laboratory* and *Wharton School*. The bigger the matrix is, the higher the inconsistency level will be (Permadi, 1992). Matrix Random Index can be seen in table below.

Table 1 Average Random Consistency Index (RI) (Permadi, 1992)

N	1	2	3	4	5	6	7	8	9	10
RI	0	0	0,58	0,90	1,12	1,24	1.32	1,41	1,45	1,49

AHP finally set out the priorities of the alternatives and the weights of each criterion with respect to the Physically land suitability map. GIS technology is used for analyzing information about development characteristics and requirements of green beans. Final thematic map is important for decision-making and prediction of land suitability, accordingly to specific crops, that are green beans ones.

Results and discussion

Agricultural crop land suitability is one of the interdisciplinary approaches that involve integration of criteria from different branches of science. Criterion may be both qualitative as well as quantitative and are involved in analysing different alternatives. Decisions have to be taken at various levels starting from selecting the land utilization types or crops till the allocation of the land utilization types or crops for area that suit best. So the suitability evaluation is a multiple criteria decision making process

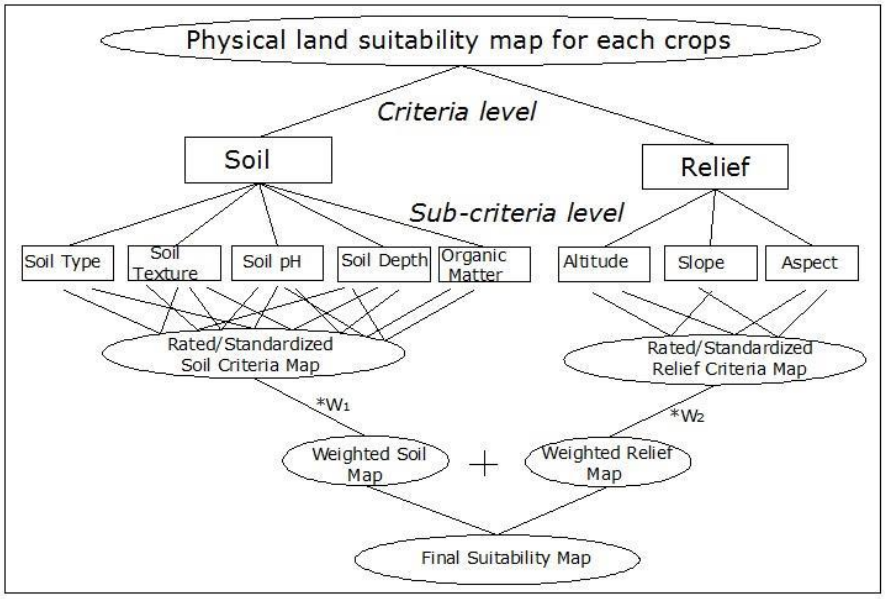


Figure 1. Conceptual model of AHP technique in GIS application

AHP technique presented the structuring of the criterias and sub-criterias required for the land suitability necessary for green beans development and set them in a hierarchical form. The overall goal of the research is suitability evaluation which occupies the top most level in the hierarchy. The next level consists of the main criteria set out to support the goal, and sub-criteria of the criteria occupy position in the next hierarchical level. Such structure allows the incorporation and accommodation of both qualitative and quantitative criteria for assessing land suitability.

Step on the research of assessment rate of soil and relief characteristics for green beans, named “Creating of land assessment database for vegetable crops in Plovdiv Region, Bulgaria” by authors Zh. Arnaudova, V. Stefanova, D. Haytova, comparing between database using matrix structure can be done. Hierarchical organization is followed by the comparison in pairs with respect to each factor of the next higher level. It allows expressing the comparisons in verbal terms which are then translated in the corresponding numbers. The next two tables present sub-classes of soil characteristics and relief ones, using matrix pair-wise comparison.

Table 2: Pair wise comparison matrix of Soil criteria in AHP

Factor	soil type	soil texture	organic mater	Ph	soil depth
soil type	**1**	0,2	0,167	0,333	0,167
soil texture	5	**1**	1	3	0,25
organic matter %	6	1	**1**	3	0,333
Ph	3	0,333	0,333	**1**	0,333
soil depth	6	4	3	3	**1**
∑	21	6,533	5,503	10,336	2,083

Table 3: Pair wise comparison matrix of Relief criteria in AHP

Factor	altitude	slope %	aspect
altitude	**1**	0,25	0,143
slope%	4	**1**	0,25
aspect	7	4,0	**1**
∑	12	5,250	1,393

Using matrix for presenting compare between every one sub-criteria in value, we transform all information into numbers and put it on GIS tools. For achieving weight assessment of land parameters, all values have to be calculated by using the above mentioned formulas. The results are in the next two tables.

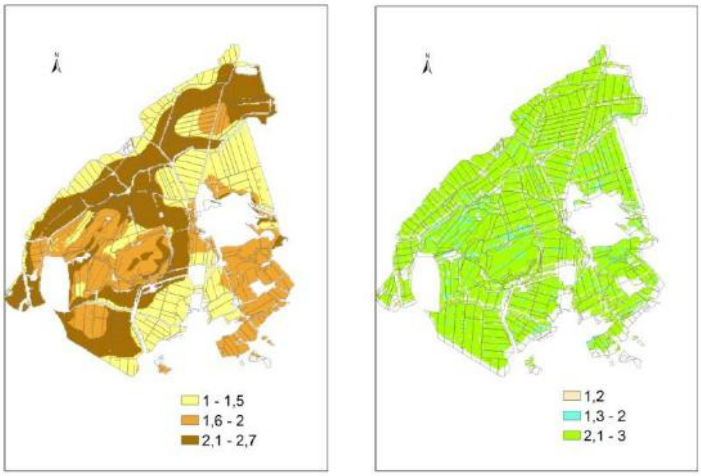
Table 4: Weighted parameters of Soil criteria in AHP

Factor	soil type	soil texture	organic matter	Ph	soil depth	Total	Average	consistency measure
soil type	0,048	0,031	0,030	0,032	0,080	0,221	0,044	5,142
soil texture	0,238	0,153	0,182	0,290	0,120	0,983	0,197	5,335
organic matter %	0,285	0,153	0,182	0,290	0,160	1,070	0,214	5,277
Ph	0,143	0,051	0,061	0,097	0,160	0,511	0,102	5,078
soil depth	0,285	0,612	0,546	0,291	0,480	2,214	0,443	5,520
							CI	0,068
							RI	1,12
							CR	0,06

Table 5: Weighted parameters of Relief criteria in AHP

Factor	altitude	slope%	aspect	Total	Average	consistency measure
altitude	0,083	0,048	0,103	0,234	0,078	3,015
slope %	0,334	0,190	0,179	0,703	0,234	3,062
aspect	0,583	0,762	0,718	2,063	0,688	3,156
					CI	0,039
					RI	0,58
					CR	0,07

Giving priority for every one of the physical environment criteria is calculated and based on expert knowledge and farmer opinions. Local conditions assessment and crops requirements were also made use for this. Accordingly to the expert opinions, the soil characteristics are more imported than relief parameters. Accepted it weight for these two parameters are 80% for soil and 20% for relief. Rated criteria maps are combined with the weightings to provide suitability value for each land unit corresponding to green beans. The score of the criteria obtained from the AHP will be stored as attribute data of each of the land unit of study area. ArcGIS is used to combine spatial data with suitability index so that a continuous land suitability map is generated. Final thematic maps are created and edited, overlaid and visualized on ArcGIS software of ESRI. The results from calculated parameters are presented in Soil and Relief thematic maps.



(a) weighted soil map *(b) weighted relief map*

Figure 1: Land assessment rate of Soil and Relief parameters for *Phaseolus vulgaris* L. in Perushtitza Village

Figure 2: Physical Land Suitability Map for *Phaseolus vulgaris* L. in Perushtitza Village

Once the weight value assigned for each class of each map, all of maps of factors for each land use type will be multiplied together. This study conducted calculations of the weight and fact value of each criteria and sub-criteria, produced the theme layers of each subcriteria, overlaid all the theme layers for having the final suitability classification. The thematic map with suitability value will be generated for every one level and overlaid for final suitability classification accordingly to the study area. As a result the most suitable soil characteristics in the Perushtitza Village are presented with best rated.

Multidisciplinary natural resources teams are required to make GIS systems an effective tool in support of land evaluation and land use planning. Each map demonstrates information, spatial and non-spatial attributive relating to land evaluation objective. Good and positive land assessment of physical parameters in Perushtitza Village is established for green beans (*Phaseolus vulgaris* L.). This state is proven by employing GIS technique. Using AHP method of dealing is complex decision making and can assist with weighting selection criteria, analysing the data collected for the criteria, and expediting the decision-making process. All this information can be beneficial for predictions, planning and getting more increasingly profitable yield by green beans growing.

Conclusion

The aim of land evaluation is to determine the suitability of land for alternative, actual or potential, land uses that are relevant to the area under consideration. Land evaluation is the process of predicting the use potential of land on the basis of its attributes. It is interdisciplinary activities that rely on large amounts of information from different sources. Finally the results with reports and maps have to be dynamic, considering the continuous refinement of the whole land evaluation process. The results are intended to be used for land resource related decision making, both strategic land use planning by the direct land users, that is, the farmers.

Acknowledgement

The report and participations on the congress was financial supported by Project 05-14 of Research Centre in Agricultural University, Plovdiv (Bulgaria).

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Original scientific paper
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CREATING OF LAND ASSESSMENT DATABASE FOR VEGETABLE CROPS IN PLOVDIV REGION, BULGARIA

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Abstract

Development of vegetable crops is height and increased procession in Bulgaria. Plovdiv region is special area because of its green beans production. The main purpose of this report is investigating and analysing some of important factors for sustainable vegetable cultivation, especially green bean (*Phaseolus vulgaris* L.) . The volume of data include information about biological requirements for cultivation: climate, climatic conditions, terrain and soil characteristic, monitoring and agricultural facilities, socio-economic conditions like existing irrigation systems, road systems, mechanization, production transporting, etc. Gathering date from different kind of sources and then presented it on tables, makes work and analyzing easier to be used. Transform all non-spatial information into spatial using computer technology can be made by GIS applications. All information is prepared for using of GIS application by converting it into spatial database. According to specify crop requirements and surrounding environment, GIS analyzing presented more hopefulness and predicted way to show dependence to vegetable characteristics on nature. Land parameters and climate characteristics are part of the physical land characteristics, necessary for vegetable growing. All information is collected from different kind of resources- experiences, researches, literatures, etc. This report emphasize on physical land characteristics and especially soil and relief parameters, which is more important for crops development. About soil characteristics are discussed soil type, soil texture, organic matter, soil pH and soil depth. Accordingly to relief are presented altitude, slope and aspect. Their dependency and spatial transformation are presented by thematic maps

Key words: *vegetable crops, GIS, assessment, spatial database.*

Introduction

Horticulture is one of the most approached cultivation branch in Bulgaria country. Establishing appropriate suitability factors is the construction of suitability analysis. Careful planning of the use of land resources is based on land evaluation, which is the process of assessing the suitability of land for alternative land uses (Fresco et al, 1994). Information on land resources is a key to their careful and effective evaluation. Farming systems involve a complex combination of inputs, managed by farm households but influenced by environmental, political, economic, institutional and social factors. Good knowledge of crop necessaries is one of the steps to be good and profitable farmer. Among the horticultural sub-sectors, vegetable production increased at the fastest rate, especially during the last decade. High rate of vegetables depends on necessaries of the people and natural resources. Most horticultural commodities are commercially produced for the market (except from home-garden), which creates substantial demand for marketing activities.

The aim of this research is to create database for land assessment and analysing some of important factors for sustainable vegetable culture, especially green bean (*Phaseolus vulgaris* L.) Land parameters and climate characteristics are part of the physical land characteristics, necessary for vegetable growing. All information is collected from different kind of resources- experiences, researches, literatures, etc. This report emphasize on physical land

characteristics and especially soil and relief parameters, which is more important for crops development (Kumanov, 1988). About soil characteristics are discussed soil type, soil texture, organic matter, soil pH and soil depth. Accordingly to relief are presented altitude, slope and aspect. Their dependency and spatial transformation are presented by thematic maps.

Study area

Study area is Perushtica Village, Plovdiv Region, Bulgaria where topography, slope, aspect, etc develop considerable difference in the micro-climatic regime within study area the land unit can carefully be delineated. This study includes information from the climate, meteorology and environmental characteristics of the Perushtitza Village, Bulgaria. This region covers 4871.6 ha, including 2298.9 ha land using area. Elevation changes from 150m to 800 m. The relief is various from plane to hilly. The urban part is situated on plane and agricultural land. There is concerned most of the useful area for vegetable growing (Arnaudova, 2011, Popov 2011).

Figure 1. Study area

Material and method

The research needed information on physical land characteristics - climate, topography, soil, land cover and land use. In addition to these, data were collected to assess indicators of land assessment suitability. All necessary information is complex characteristics and depends on biologically requirement of vegetables.

Geographic information

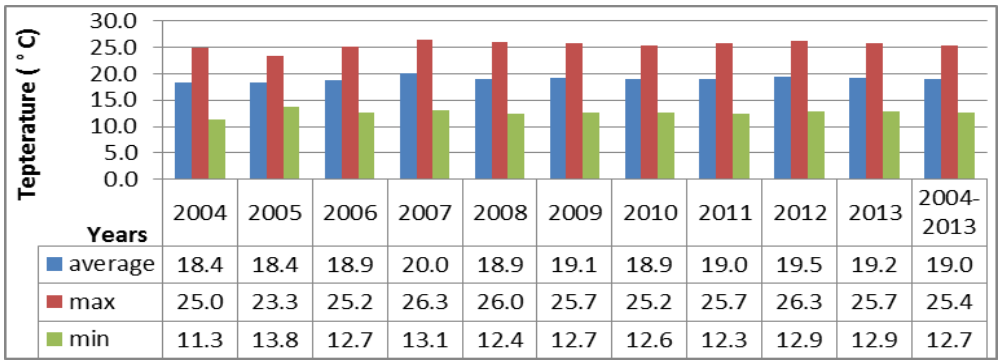
Digital administrative maps of the region, municipality, digital cadastral maps and maps of reclaimed property in the studied area. The digital model formats are ZEM, CAD. Information source: the Geodesy, Cartography and Cadastre Agency. (Arnaudova, 2010)

Digital soil map of the area in scale M 1:10000. Soil maps reflect in detail the boundaries between the separate soil types. Information source: The Soil Resources Agency and the Institute of Soil Science “Nikola Pushkarov”.

Topographic maps in scale M 1:25000 and digital elevation models

Attribute information

Air temperature in °C for period of 10 years (2004-2013), especially the months from April to October. This period is enough to establish dependence between variety of maximum, minimum and average temperatures.



Years	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2004-2013
average	18.4	18.4	18.9	20.0	18.9	19.1	18.9	19.0	19.5	19.2	19.0
max	25.0	23.3	25.2	26.3	26.0	25.7	25.2	25.7	26.3	25.7	25.4
min	11.3	13.8	12.7	13.1	12.4	12.7	12.6	12.3	12.9	12.9	12.7

Figure 2. Distribution of air temperature for period of 10 years (2004-2013)

Crop requirements for green bean (*Phaseolus vulgaris* L.) are presented on Table 1. It includes one of the most important characteristics, on which depends good vegetables development.

Table.1 Crop requirements for suitability of green bean (*Phaseolus vulgaris* L.)

Parameters	Potential Ratings		
	High	Moderate	Low
Temperature (° C)	22 -25	15 -22, 25 -28	< 10 & > 30
Soil texture (%)	25-30	30-40	>40
Organic matter content (%)	>2,5	1.5-2	<1.5
Soil reaction (pH)	6.6 - 7.5	< 4.5-6.6 & 7.5 >8.2	
Irrigation	Regular	Partly available	Rained
Soil depth (cm)	>80 cm	50-80cm	<50cm
Slope (degree)	Flat to 1	1 - 5	5 - 8
Aspects (direction)	South	South East	North
Soil Fertility	High	Moderate	Low

Assessment rate

In land suitability analysis, a map represents each evaluation criterion with alternatives (S1, S2, S3) indicating the degree of suitability with respect to a criterion. These classes have to be rated, how important is the class S1 with respect to a particular criteria to contribute for the final goal (suitability). In this particular land suitability analysis the criteria are mainly related to climate factors, elevation structure, soil characteristics. The next table explains suitability criterions.

Table 2. Suitability rate

Suitability classification	Explanation
High suitable (S1)	Suitable capacity of locations is high and satisfies all criteriaset up.
Medium suitable (S2)	Suitable capacity of locations is medium and satisfies of the criteria set up, but some criteria are not satisfied.
Low suitable (S3)	Suitable capacity of locations is low and satisfies some of the criteria set up, but most of the 2criteria are not satisfied.

The methodology is based on matching soil/land characteristics against agronomic requirements of crop and then the suitability classification will be assessed. The selection of criteria is the crux of the suitability analysis. Physical land suitability evaluation is based on biophysical conditions of the study area. The process of selecting the main criteria and sub-criteria is iterative in nature. Literature review, analytical study and the local opinions were basic tools for selection of evaluation criteria (Baniya, 2008).

Results and discussion

All collected information is classified by suitability rate and prepared for using of GIS applications. Using suitability classification for land assessment, gathering data can be presented by thematic maps, using GIS tools

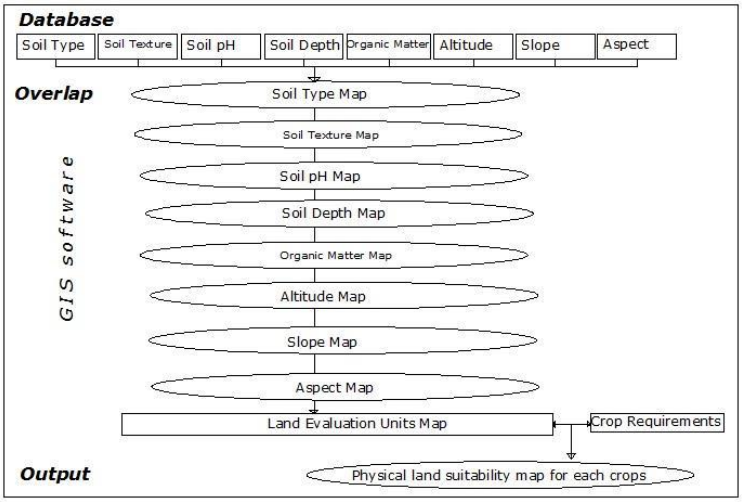
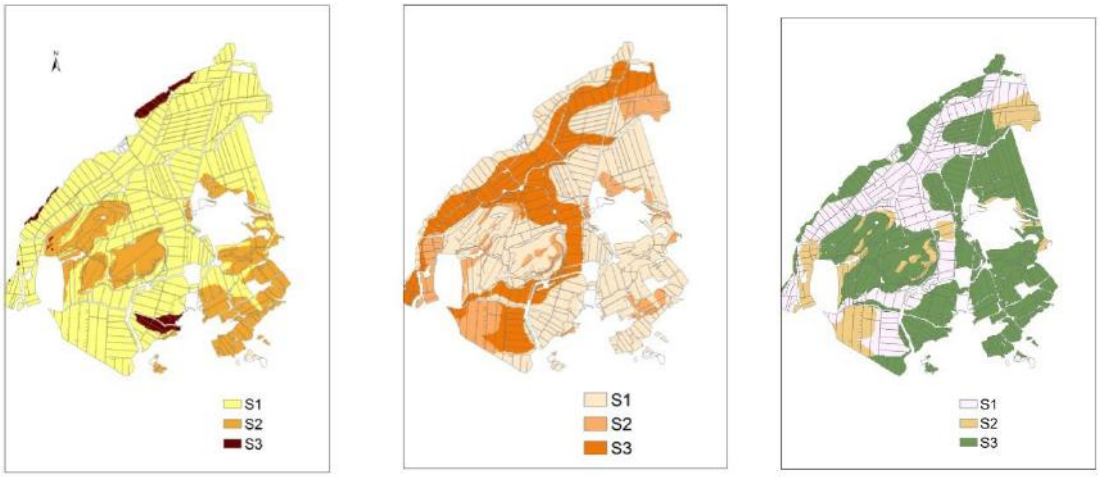
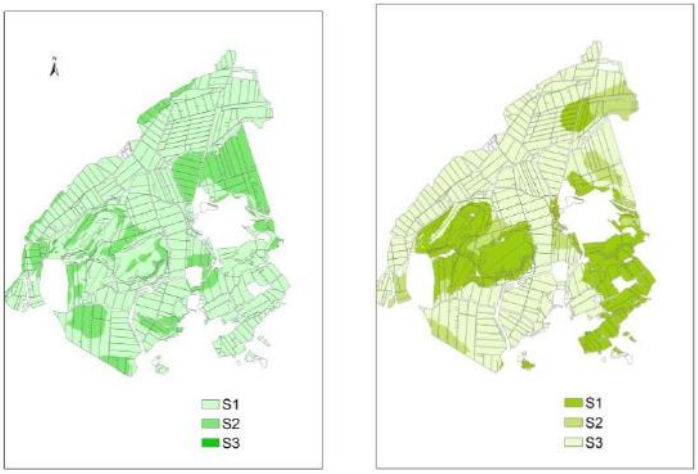


Figure 3. Conceptual model for assesment physical land suitability

Research methods include collection of available and new field work data, data analysis using various tools and techniques. Several sources were used to gather up all data.

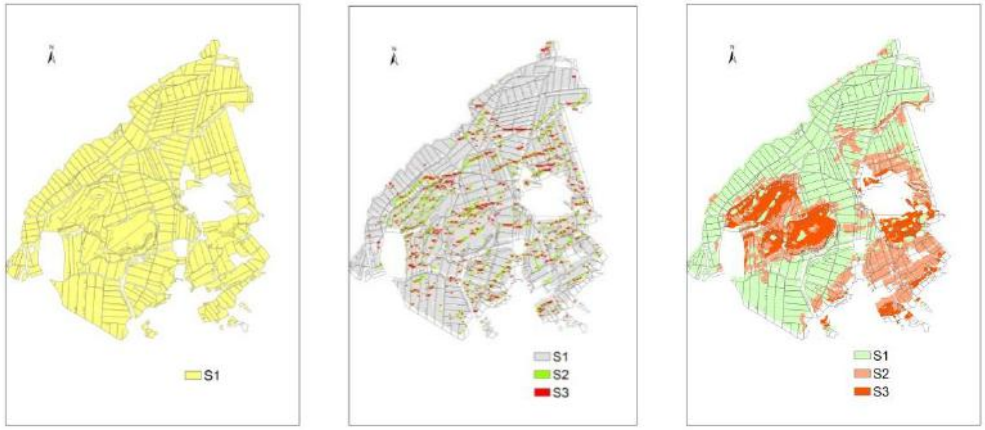


(a) soil type (*b) soil texture* *(c) soil depth*



(d) Reaction of soil (Ph) *(f) Soil organic mater*

Figure 4. Suitability soil characteristics maps in Perustitza Village for (*Phaseolus vulgaris* L.)



(a) elevation *(b) aspect* *(c) slope (%)*

Figure 5. Suitability topographic characteristics maps in Perustitza Village for (*Phaseolus vulgaris* L.)

Land suitability evaluation is needed for various purposes in the context of present day agriculture and has to be carried out in such a way that local needs and conditions are reflected well in the final decisions.

Most spread soil types in the region are suitable for the vegetable cultivation. Respecting the soil physical characteristic - soil texture and soil depth are differently. Texture is one of the important parameter of soil. Most of the physical characteristics of soil depend upon texture class. (*Phaseolus vulgaris* L.) prefers loamy to sandy loam soil. Requirements for rootable soil depth is minimum 60 cm. These soils are about 40 % of whole cultivated area in region.

Organic matter content of the soil is an important parameter related to soil fertility. The most organic matter is ranging to 2% or medium suitable for vegetables. Figure 3 (a), (b), (c), (d), (f).

Refers to the climatic and infrastructure characteristics of studied area, the suitability rate can be defined to the S1- high suitability region for green bean growing. This assessment can be useful for more farmers and profitable for marketing centres. Figure 4 (a), (b), (c).

Conclusion

The most of cultivated area in Perushtitza Village are suitable for green bean cultivation. More of the physical land characteristics fitted on crop requirements with best rated.

Making suitability thematic maps the prediction and planning of profitable yields is more accessible to the farmers.

The selection of the main criteria and sub-criteria for each soil and topographic characteristics are first step to suitability evaluation process. Assessment of socio-economic and environmental suitability evaluation is carried only on those areas which are physically suitable.

Land evaluation is part of the process of land-use planning and the results should be useful for rational land use planning. Land use planning is a tool to help policy makers, decision makers, and land users, to use land in a way, that current land use problems are reduced and specified, social, economic or environmental goals are achieved.

Acknowledgements

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VERTICAL DISTRIBUTION OF SOIL SALINITY IN NERETVA RIVER ESTUARY

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Abstract

The goal of this research was to establish vertical distribution of soluble salts in ameliorated soils of Neretva River estuary located in the Adriatic Sea coast of Croatia. In the study area covering 670 ha in central part of estuary 77 soil pits were excavated. Each soil profile was segmented in 4 depth zone (0-30, 30-60, 60-90 and 90-130 cm) from which soil samples for laboratory analysis were collected. In a total of 308 soil samples basic soil chemical properties (pH, humus, $CaCO_3$, P_2O_5 and K_2O), soil texture and electrical conductivity (EC), Cl^-, SO_4^{2-}, Na^+, Ca^{2+} and Mg^{2+} in 1:1 soil:water extract were determined. Investigated ameliorated hydromorphic soils developed on delta sediments (quartenary marine mineral and organic deposits and alluvial deposits) had very wide ranges of analyzed chemical properties and texture. Soil conductivity ($EC_{1:1}$) varied in wide range from 0.42 - 8.00 dS/m (non-saline to strongly saline soil). Average soil $EC_{1:1}$ in surface and sub-surface horizons (depth zone 0-30 and 30-60 cm) were 2.06 and 2.38 dS/m respectively what makes them slightly saline. Deeper depth zones 60-90 cm and 90-130 cm were moderately saline with average values of soil EC 3.05 and 3.34 dS/m. Investigation of vertical distribution of soil salinity showed that soils of this intensively agro-exploited areas (citruses, other fruits and vegetables) are exposed to degradation: primary salinization caused by the capillary rising of groundwater and secondary salinization by using of brackish water for crop irrigation.

Keywords: *soil, salinity, agriculture, amelioration*

Introduction

Soil salinity of the irrigated land in the Neretva River estuary are result of primary or natural soil salinity processes (i.e. intrusion of highly salinized water in land) and secondary or anthropogenic (irrigation with brackish water, application of inorganic/organic soil supplements, fertigation). The influence of the salinization processes in Neretva River estuary was studied in numerous studies (Ondrasek et al., 2011; Romic et al 1999, Klačić et al, 1998; Salopek, 2008) and suggests their potentially large influence on the irrigated agriculture land. The intensive rainfall between two growing seasons and favorable soil properties (light texture and good water permeability) enable leaching of accumulated salts from rhizosphere into deeper soil layers and thus ensure the sustainability of the agricultural system. Soil salinization processes are very dynamic and may lead to problems for agriculture production so their consequences should be monitor. Previous investigations mainly analyzed horizontal distribution of salt in topsoil, as well as irrigation water quality, while data for vertical distribution of salt in soils of this area are missing. The goal of this research was to establish vertical distribution of salts in ameliorated soils of central part of Neretva River estuary.

Materials and methods

Study area

The study area is located in the Neretva river estuary in the Adriatic Sea coast of Croatia (43^0 $01^{'}$ $42^{"}$ E and 17^0 31' 10"N) covering 670 ha of agricultural land (Fig. 1). According to Köppen (1918) climate classification researched location belong to the climate type Csa, which means

that the area has a Mediterranean climate with hot and dry summers and wet winters. The mean monthly temperature was 15.7°C, and mean annual precipitation was 1231 mm (meteorological station in Opuzen, 1981-2000.) The climatic conditions are suitable for growing Mediterranean crops and vegetables. Geologically, terrain consists of the delta sediments (Marinčić et al. 1971) composed of Quaternary alternately accumulated marine, freshwater and lagoon sediments. The bed of the Neretva River at the mouth is a very deep (9 to 11 meters) and therefore allows the penetration of sea water upstream, especially during the low water levels of the Neretva River. This is especially noticeable in the summer, particularly during high tide. Before amelioration in the 60's of the 20th century this location was typical brackish marsh environment. The terrain elevations of study area ranging between -0.50 and 0.5 m. Today, the area is protected from flooding and the raising of brackish water in the channels by the pumping system. This area is used for growing vegetables, citruses and other fruits. Using WRB classification (FAO 2006), soils are classified as Fluvisols Calcaric and Molic and Gleysols Molic hydroameliorated by channels. Due to the degree of salinity these soils are classified as non-saline, salinized and saline soils.

Figure 1. Location map of the study area

Soil sampling and chemical analyses

In the study area covering 670 ha in the central part of Neretva River estuary 77 soil pits were excavated. Each soil profile was segmented in 4 depth zone (0-30, 30-60, 60-90 and 90-130 cm) from which soil samples for laboratory analysis were collected. A total of 308 soil samples were taken. On collected soil samples laboratory analysis were carried out by standard methods (JDPZ, 1966): pH potentiometrically, humus content by Kotzman method, total carbonate content by volumetric method (Scheibler), content of the available phosphorus by Troug method, potassium by AL method and texture by International B pipet method. Electrical conductivity (EC) was determined in 1:1 soil to water extract conductometrically, chlorides by Mohr method, sulfates by turbidimetric method, sodium by flame photometric method, Ca and Mg by AAS method.

Results and discussion

Statistical parameters of basic chemical properties and soil texture are shown in the Table 1. Investigated soils are weakly acid to alkaline, in average alkaline in all horizons. Deeper horizons, below 60 cm have higher pH regarding upper horizons. Coefficients of variation (CV) are small (2.62-3.61%) and a frequency distribution is symmetrical. Investigated soils are slightly to strongly carbonated in all horizons. The average carbonate content increases

with depth from 25.17% in depth zone 0-30 cm to 39.34 % in zone 90-130 cm, what is in agreement with above described increasing of pH values with depth. The average humus content decreases from 4.71% and 4.46% in the first two depth zones (0-30 and 30-60cm) to 2.93% and 2.72% in the third and fourth depth zone (60-90 and 90-130cm). Analyzed soils had a wide range of humus content in all depth zones, CV with a depth increasing from 42.09% in first depth zone to 83.24% in the fourth depth zone (90-130 cm). A frequency distribution of the humus content in first two depth zones is symmetrical. An asymmetrical distribution with a long tail to the right (higher values and positive skew) has the humus in the third and fourth depth zone. Depth zones 0-30 cm and 30-60 cm is very poorly to very richly supplied with available potassium. In average, depth zone 0-30 cm is richly supplied and zone 30-60 cm well supplied with K_2O. Depth zone 0-30 and 30-60 cm are averagely very poorly supplied with available phosphorus, although it vary from very poorly to slightly supplied in surface horizon. Very high CV, as well as asymmetrical frequency distribution (higher values and positive skew in both horizons) indicates differences in land management (fertilization).
According to texture the first three depth zones are averagely silty loam. The fourth depth is loam, with very variable content of coarse sand and clay (CV 144.23% and 68.00% respectively), as well as asymmetrical distribution (skewness 1.99 and 1.18 respectively). The average clay content decreases with depth from 25.06% (zone 0-30 cm) to 12.04% (zone 90-130 cm.). The most variable fraction in all depth zones is coarse sand with CV from 130.82% (zone 0-30 cm) to 187.39 % (zone 60-90 cm) and asymmetrical distribution in all depth zones.

Table 1: Statistical parameters of basic chemical properties and soil texture

Stat.	pH		$CaCO_3$	Humus	K_2O	P_2O_5	Coarse sand	Fine sand	Silt	Clay
	H_2O	KCl	%		mg/100g.		2-0.2	0.2-0.02	0.02-0.002	<0.002
Depth zone: 0–30 cm										
Mean	7.73	7.28	25.17	4.71	32.62	1.87	3.77	34.34	36.81	25.06
Min	7.20	6.90	3.20	1.31	5.50	0.10	0.10	10.50	13.70	3.40
Max	8.20	7.64	51.70	10.11	75.00	8.00	20.80	61.10	59.00	42.90
CV%	2.62	2.33	43.75	42.09	43.50	81.35	130.82	37.87	25.34	33.42
Skewness	-0.14	-0.18	0.16	0.63	0.69	1.96	1.85	0.30	-0.20	0.04
30-60 cm										
Mean	7.70	7.27	26.85	4.46	25.80	0.98	4.75	35.86	36.44	22.96
Min	6.80	6.35	3.10	0.61	2.50	0.10	0.00	13.90	4.60	5.50
Max	8.32	7.97	54.40	10.06	67.00	5.00	49.80	75.10	62.80	43.70
CV%	3.61	3.62	43.46	50.21	52.87	98.48	171.76	42.33	32.62	39.34
Skewness	-0.61	-0.61	0.14	0.50	0.65	1.73	3.12	0.61	-0.35	0.01
60-90 cm										
Mean	7.72	7.39	30.63	2.93	-	-	6.83	44.03	30.28	18.87
Min	6.85	6.34	2.90	0.31	-	-	0.00	7.70	2.00	1.20
Max	8.30	8.24	57.00	10.00	-	-	81.40	80.90	70.70	50.20
CV%	3.56	4.28	46.70	73.34	-	-	187.39	44.40	51.00	65.09
Skewness	-0.84	-0.74	0.00	1.37	-	-	3.55	-0.03	0.17	0.81
90-130 cm										
Mean	7.79	7.51	39.34	2.72	-	-	11.33	54.48	22.15	12.04
Min	7.00	6.50	6.20	0.38	-	-	0.00	12.30	0.90	0.40
Max	8.41	8.24	63.60	9.88	-	-	71.80	82.50	54.80	36.90
CV%	3.35	4.36	33.71	83.24	-	-	144.23	33.60	57.61	68.00
Skewness	-0.33	-0.65	-0.46	1.53	-	-	1.99	-0.47	0.91	1.18

The average electrical conductivity ($EC_{1:1}$) increases from 2.06 dS/m in the first depth zone (0-30 cm) via 2.38 dS/m in the second depth zone (30-60 cm) to 3.05 and 3.34 dS/m in the

third and fourth depth zone (60-90 cm and 90-130 cm), Table 2.A frequency distribution of the EC in first two depth zones has a symmetrical distribution. In the third depth zone distribution of frequencies is becoming close to asymmetric. An asymmetrical distribution with a long tail to the right (higher values and positive skew) has EC in the fourth depth zone.
In order to be able to interpret $EC_{1:1}$ should be converted into ECe values. Various authors used different correlation equations and factors to convert 1:1 soil: water extract to saturated paste extract equivalents: USDA (1954), Hogg & Henery (1984), Landon (1984), Zhang et al. (2005), Smith and Doran (1996) and Sonmez. et al. (2008)). In this paper we used proposal of Whitney (1998) which takes into account different texture classes. According to this proposal, in Table 2 are given the description of the salinity classes for mean, minimum and maximum $EC_{1:1}$ values of the each depth zone. First and second depth zones are slightly saline, third and fourth depth zone are moderately saline. The ranges and coefficients of variation (CV) showed the great variation and with depth increased. Degree of salinity in the first, second and third depth zone varies from non-saline to strongly saline (0.42-7.46 dS/m) and in fourth depth zone varies from slightly to moderately saline (1.33-8.00 dS/m).
The chlorine (Cl^-) and sodium (Na^+) concentrations had a wide range, a large coefficient of variation, and a symmetrical distribution in the all depth zones (Table 2). In the first depth zone Cl^- and Na^+ concentrations were 4.06 and 3.78 meq/L respectively with a tendency of a slight decline in the second depth zone. In the third and fourth depth zone the average Cl^- and Na^+ were increased and amounted 5,39 meq/L and 5,24 meq/L, respectively. Analysis of the original data showed that in 21 of the total 77 soil profiles, first depth zone has a higher Cl^- and Na^+ concentrations and higher nutrient content of phosphorus and potassium. This topsoil salt accumulation was mainly caused by the use of brackish water for irrigation.
The average sulphate (SO_4^{2-}) concentration gradually increased with depth between 18.59 meq/L in the first depth zone up to 40.92 meq/L in the fourth depth zone, with symmetrical frequency distribution. The average magnesium concentration (Mg^{2+}) increased with depth from 3.53 meq/L in the first depth up to 13.73 meq/L in the fourth depth zone. Mg^{2+} concentrations were in wide ranges in all depths zones with great CV. In second depth zone Mg^{2+} had asymmetrical distribution with a positive skew, while in other depth zones frequency distribution were close to asymmetrical (skewness 0.96-0.99). The average Ca^{2+} increased with depth, 19.96 meq/L in zone 0-30 cm to 28.29 meq/L in zone 90-130 cm. A frequency distribution of Ca^{2+} concentrations were symmetrical in first 60 cm, while in deeper depth zones were asymmetrical with negative skew (lower values and a long tail to left). Sodium adsorption ratio (SAR) vary from 0.1 to 7.3 with great CV and asymmetrical distribution with positive skew. In all depth zones SAR is below 13 what is considered limit value for sodic soils. Greater variation and higher skewness were registered in deeper depth zones (60-90 and 90-130 cm).

Table 2: Statistical parameters of EC, Cl^-, SO_4^{2-}, Na^+, Ca^{2+} and Mg^{2+} in 1:1 soil:water extract SAR and salinity classes for mean, minimum and maximum values of the $EC_{1:1}$

Stat.	$EC_{1:1}$	Cl^-	Na^+	SO_4^{2-}	Mg^{2+}	Ca^{2+}	SAR	Salinity classes*
	dS/m			meq/L				
Depth zone 0-30 cm								
Mean	2.06	4.06	3.78	18.59	3.53	19.96	0.99	Slightly saline
Min	0.42	0.10	0.10	0.20	0.30	2.70	0.10	Non-saline
Max	5.05	12.50	12.50	41.70	9.90	43.40	3.40	Moderately saline
CV %	53.78	91.46	84.09	69.96	72.64	58.58	88.14	
Skewness	0.18	0.86	0.82	0.20	0.98	-0.01	0.98	
30-60 cm								
Mean	2.38	3.94	3.65	27.67	4.63	23.87	1.20	Slightly saline
Min	0.55	0.10	0.10	2.10	0.50	3.80	0.10	Non-saline
Max	5.62	12.60	9.80	50.00	15.60	36.00	5.10	Moderately saline
CV %	40.59	89.09	80.11	46.59	69.11	46.78	99.03	
Skewness	0.13	0.69	0.46	-0.66	1.14	-0.83	1.57	
60-90 cm								
Mean	3.05	4.55	4.35	35.72	9.07	27.41	1.24	Moderately saline
Min	0.90	0.10	0.10	1.90	0.90	2.50	0.10	Non-saline
Max	7.46	12.80	14.50	166.70	28.80	40.50	6.80	Strongly saline
CV %	41.95	79.65	87.68	58.43	71.06	30.83	107.22	
Skewness	0.90	0.64	0.99	0.42	0.96	-1.38	1.91	
90-130 cm								
Mean	3.34	5.39	5.24	40.92	13.73	28.29	1.29	Moderately saline
Min	1.33	0.10	0.10	8.30	1.90	8.40	0.10	Slightly saline
Max	8.00	15.20	15.12	81.70	37.80	36.00	7.30	Strongly saline
CV %	42.28	73.65	78.14	31.44	71.43	20.88	112.68	
Skewness	1.78	0.72	0.47	0.62	0.99	-1.77	2.27	

*Salinity classes according to Whitney (1998)

Relationships between soil texture classes and electrical conductivity ($EC_{1:1}$) are shown in Table 3. According to LSD test at 5% confidence interval difference of the $EC_{1:1}$ in sandy soil texture classes are statistically significant different (lower) in all depth zones regarding heavier texture classes. The absolute differences between loamy and clayey texture classes were not statistically significant.

Table 3. The mean $EC_{1:1}$ values of the different soil texture classes

Depth	Texture		
cm	Sandy	Loamy	Clay
0-30	1.75^b	2.12^a	2.15^a
30-60	2.02^b	2.59^a	2.44^a
60-90	2.59^b	3.48^a	3.43^a
90-130	3.05^b	4.34^a	4.12^a

Labels $^{a, b}$ according to LSD test at 5% confidence interval

Conclusion

Electrical conductivity evaluated using 1:1 soil to water extract ($EC_{1:1}$) varied over a wide range (0.42-8.00 dS/m), so soils were classified as non-saline, salinized and saline. Vertical distribution of the electrical conductivity ($EC_{1:1}$) is characterized by a gradual increase from 2.06 and 2.38 dS/m in the first and second depth zone (0-30 and 30-60 cm) to 3.05 and 3.34 dS/m in third and fourth depth zone (60-90 cm and 90-130 cm). A similar vertical distribution (gradual increase with depth) has sulphate ion. The chlorine (Cl^-) and sodium (Na^+)

concentrations in the first depth zone were 4.06 and 3.78 meq/L respectively, with a tendency of a slight decline in the second depth zone. This topsoil salt accumulation was mainly caused by the use of brackish water for irrigation. In the third and fourth depth zone the average Cl^- and Na^+ were increased and amounted 5.39 meq/L and 5.24 meq/L, respectively. The results showed that primary salinization caused by the capillary rising of groundwater and secondary salinization by using of brackish water for crop irrigation is present in Neretva River estuary. The sustainability of this agro ecosystem, is based on the intensive rainfall and favorable soil properties, especially light texture and good water permeability that ensure leaching of salts from the root zone to deeper soil horizons.

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AN EXPLORATORY SURVEY ON HOUSEHOLD FOOD WASTE IN EGYPT

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Abstract

Food security is a major concern in the developing countries, food production must increase significantly to meet future demand in a way that assures balance between the available and limited natural resources. According to the FAO roughly one-third of the edible parts of food produced for human consumption gets lost or wasted globally, i.e. about 1.3 billion tons per year. Food losses and waste are generated through the whole food chain.

Changes in food consumption patterns in Egypt had implications also in terms of the amount of food lost and/or wasted. Unfortunately, there are few data regarding food waste in Egypt. Therefore, the current exploratory study aims to have a general overview about household food waste in Egypt. An online survey and face-to-face interviews were conducted from February to May 2015 with a random sample of 181 adult Egyptians representing about 64.6% female and 35.4% male. The majority of the respondents were young (59.1% are less than 44 years old) and have high education level. Food waste is prevalent in Egypt as just 13.8% of respondents declare that they do not throw any food. Data show that food waste increases during the fasting month of Ramadan. The most wasted food products are fruits, vegetables, cereals and bakery products. Only 21.5% of respondents declared that the economic value of food waste generated each month is more than 6US$. Most of Egyptian respondents have a good understanding of food labels that is probably due to the high education level of the sample. About 42% of respondents throw weekly at least 250 g of still consumable food. To reduce food losses and waste in Egypt it is important to set a strategy at all food chain levels. There is also an urgent need to raise people's and organizations awareness towards this problem and further exploration on food waste at lower educational levels and poor people, which might be quite different.

Keywords: *Household Food Waste, Behavioral Change, Survey, Egypt*

Introduction

Food waste takes place at two levels; retailers and consumers (Gustavsson *et al.*, 2011). At the retailers' level food is wasted due to various reasons *i.e.* poor storage facilities, while at the consumers' level it is related to purchasing behavior and attitudes, knowledge regarding labeling information, insufficient purchasing planning. Additionally, economic, social and environmental factors could affect food losses and waste (FLW), as small scale farmers might lack cash to face production costs, climate change either high temperatures or freeze, or for social reasons i.e. marriage of sons or daughters.

FAO alerted about increased rate of food losses and waste in Egypt which reaches about 50% in vegetables and fruits, 40% in fish, 30% in milk and the amount of wheat losses and waste reaches 1.5 million tons/per annum. Additionally, wheat, tomato and orange losses and waste cost the government about 11 million EGP/annually, and about 650,000 tons of maize besides 350,000 tons of beet are wasted annually. Food security problem will not be solved via

increased production only but by conserving produced food from waste and damage (Egypt time science, 2015).
Egypt has two main sub-systems for food subsidy, first wheat flour and bread subsidy, second ration card program which provides fixed monthly quota of some commodities. This subsidy system suffers from increasing budget and waste, as waste occurs at different stages of bread supply chain starting from pre-post wheat harvest, storage, transportation, conversion of wheat to flour until consumption (Ramadan, 2014).
To authors knowledge, there are no direct policies or action plans at the government level that deal with food waste. In the Ministry of Agriculture and Land Reclamation there is the agricultural strategy 2030 that deals through its programs and action plans with food safety and agriculture waste. In the Agricultural Research Centre there are some institutes that tackle the issue of food safety within research context *i.e.* Food Technology Research Institute, Food Safety Information Centre, Economic Research Institute.
Food Technology Research Institute for instance conduct applied researches in food processing and preserving to improve the quality of food products and produce safety high nutritional value food with affordable prices. In addition to utilization of agricultural wastes and food processing factories wastes to reduce the pollution and to increase the economical returns through value added food. Finally it is necessary to reduce the loss during food processing (Food Technology Institute, 2015).
Ministry of Supply and Internal Trade provides food subsidy programs, yet no food waste reduction programs, although there is a consumer protection agency. The ministry aims at improving 164 wheat barns or silos "Shownaa", in order to reduce wheat losses and waste, recycling food oil waste, applying a system for recycling household food waste and leftovers to fertilizers (Ministry of Supply and Internal Trade, 2015). In Ministry of Industry and Foreign Trade, there is Law No. 67 of 2006 that is concerned with consumer protection (Ministry of Industry and Foreign Trade, 2015). There was an attempt in 2011 to establish the National Authority for Food Safety, but was not completed.
In spite of this, few NGOs initiated their own programs that care about food waste i.e. Egyptian Food Bank (EFB) provides some schemes to feed poor people and at the same time urged hotels and restaurants to re-use all food waste (leftovers) and re-pack it in order to be distributed to those who suffer from food insecurity and as one of the means to reduce hunger. EFP has three pillars to end hunger, first, anti food wastage awareness, by holding awareness programs for individuals, hotels and restaurants in order to save the food wasted everyday from homes or from buffets in events and occasions; second, awareness for hotels and restaurants, EFP signed a protocol with the Egyptian Hotel Association in order to save the excess untouched food from hotel events' buffets and restaurants by packing it in foil trays to be distributed to the nearest NGO, elderly residence or orphanage in the area instead of throwing it away; third, awareness for individuals, EFB directed campaigns to individuals at their homes, to distribute the excess untouched food to the nearest needy (Egyptian Food Bank, 2015).
Recently, there are some initiatives at individual household levels that pack any food leftovers or waste and distribute it to poor people; also some charity religious organizations (Masjid) that provide food in the Holy Month Ramadan to poor people as well. Additionally, an association named "Egyptian Chefs Association" organized with others a conference about "The Farm to Fork" that focused on three main themes, the Farm, the Kitchen and the Fork. Part one, the Farm, covered issues related to produce, from organic farming, to managing loss and keeping water usage to a minimum (Egyptian Chefs Association, 2015).
Therefore the current study is a primary attempt to explore and analyze the situation of food waste behavior and to quantify its economic value due to lack of such studies in the Middle East and North Africa Region (MENA) and Egypt in particular.

The present study mainly aims at evaluating the household food waste in Egypt focusing in particular on food purchase behavior and household food expenditure estimation, knowledge of food labeling information, attitudes towards food waste, quantity and value of food wasted, extent of household food waste, and, finally, willingness to behavioral change for food waste prevention and/or reduction.

Materials and Methods

In order to fulfill the current study's objectives, literature review from various resources *i.e.* reports, researches, databases were used. Additionally, a survey using a structured questionnaire that was adapted to the Mediterranean context from previous questionnaires and studies on food waste provided in English and Arabic languages in December 2014 and was uploaded using social media websites *i.e.* Facebook and emails, and a number of questionnaires was collected using face-to-face interviews. The questionnaire was available online from February till the end of May 2015 and the participation was entirely volunteer.

The questionnaire consisted of 26 questions. It included a combination of one option and multiple choice questions. It was developed into 6 sections that reflects the study's objectives. In the introductory part of the questionnaire the concept of food losses and waste was introduced to inform the respondents.

From 207 questionnaires received, 26 of them were not considered because the questions were not fully answered thus there were missing data. Therefore, the total number of the sample is 181 adult Egyptians.

Data were analyzed using descriptive statistics (e.g. means, max, min, percentages), in order to get a general picture of frequencies of variables, using Microsoft Excel.

Results and Discussion

The study's results provide an overview on the main characteristics of the respondents (profile) from one hand and fulfill the objectives from another hand.

Main characteristics of respondents

The results revealed that about 64.6% of the respondents were females and about 35.4% males, which could be attributed to the fact that most men consider food issues are part of women's responsibilities. Most of the respondents (33.1%) fell within the age category of 35-44 years and 23.8% between 45 and 54. About 44.2% were MSc/PhD holders, and 34.8% hold university degree, 17.1% technical degree. This shows that social media is accessed mainly by educated people. Additionally, 81.8% have paid work (full time or part time), and 6.6% were retired. Half of the respondents (53.6%) are married with children and 18.8% are partnered (sharing same household). As for household composition, the number of members are 4-6 persons represent about 69.1% of the total sample, 24.3% whose family is composed of 1-3 persons, this indicates that medium and big size families still dominate the Egyptians even among educated people, who were assumed to follow family planning programs.

Food purchase behavior and household food expenditure estimation

This part tackles respondents food behavior and an estimation of their food expenditures in order to understand their attitudes towards food. It was found that about 44.2% purchase their food directly from open markets and 28.7% purchase from supermarkets and hypermarkets and 23.2% purchase from mini/small markets.

In Egypt, most people buy their food from more than one place, meaning that they could buy from open markets, weekly markets in rural areas, mini markets or super markets or from farmers, but buying directly from farmers is not common as rural people might buy directly from their neighbors or from the weekly village markets and in some cases city people when travelling to other governorates might buy from road outlets or farmers selling their production on the road.

From once weekly to twice weekly respondents frequently purchase their food representing about 25.4% and 26.0% respectively from the total studied respondents. Some respondents buy everyday (17.7%) and those who buy every two days represent also 17.7%, others prefer to buy the basics on monthly basis (6.6%). That could be attributed to poor planning when purchasing food even if the results showed that about 44.2% of the respondents use a list prior to purchasing food, but this could be because the nature of the respondents regarding their education and social status (high and medium class).

Most of the Egyptians spend noticeable amount of their wages on food, this was evident by this study and the household survey carried in Egypt by Central Agency for Public Mobilization and Statistics (CAPMAS). It was clear that about 71.3% of the respondents spend more than 630 EGP on food, even it is considered as compared with high income countries the lowest. According to CAPMAS (CAPMAS, 2014), Egyptians spend about 37.6% of their income on food and non-alcoholic beverages, of which 3.5% on bread and cereals, 11.1% on meat, 2.3% on fish and sea food, 6.0% on dairy products, 2.7% on oils and fats, 3.2% on fruits and finally 5.2% on vegetables.

Almost half of the respondents were attracted “sometimes” to food offers representing about 44.2% of total respondents who are attracted to special food offers which normally takes place at super and hyper markets or by bargaining at small shops or small and weekly markets, followed by 33.1% respondents who are attracted to such offers, even if sometimes these offers are done on items that are almost expired, but poverty have another word and action, and often people do not pay attention about expiry date.

Knowledge of food labeling information and attitudes towards food waste

The results show respondents knowledge about food labels which might eventually lead to reducing food waste among consumers and the respondents attitudes towards food waste and food habits. It was indicated that about 86.7% of the respondents understand and have knowledge about “use by” label as food must be eaten or thrown away by this date. This result could be attributed to the educational level of the respondents. Whereas only 11% regarded the “best before” label as food is still safe to eat after this date.

It was evident that 85.6% of the respondents do worry about food waste and they try to avoid it and 8.3% are aware about food waste problems but have no intension to change their current habits. About 53% of respondents indicated that they dispose “very little” amount of uneaten food, as they try to minimize household food waste since they are worried and aware about food waste impacts, and about 29.3% throw reasonable amount of uneaten food. Unfortunately, respondents indicated that food waste increase in the Holy Month of Ramadan as mentioned by 75.7% of the respondents whereas 24.3% were negative about it.

Regarding food waste management, 41.4% of the respondents give the remained food to animals, but this happens in rural areas, as well as some urban districts were people raise mainly poultry. 34.8% said they dispose it in the garbage.

Tracking consumers food habits could explain their attitudes towards food waste and its quantity, in that regard the results showed that 57.5% of the respondents cook a main meal from raw main ingredients from 3-6 times/week. About 67.4% eat a meal left over from a previous day (less than twice/week), 50.8% eat out, this if to be true is the case in urban and big cities, but in rural areas maybe the image is different.

Quantity and value of food wasted and extent of household food waste

This part of the results deals with the quantity and value of food wasted to help quantify the amount and extent of food waste. The results showed that 63.5% of the respondents spend less than 2% of their income and about 19.3% of respondents spend from 3-5% of their income on cereals and bakery products that are wasted. Whereas 75.7% of the respondents spend less

than 2% of their income on roots and tubers, 84% spend less than 2% of their income on pulses and oil seeds that are wasted (table 1).

Table 1: Respondents estimation for purchased food groups wastage (in percent).

Items	Less than 2%	3 to 5%	6 to 10%	11 to 20%	Over 20%
Cereals and bakery products (bread, rice, pasta, etc.)	63.5%	19.3%	9.9%	0.6%	6.6%
Roots and tubers (potatoes, etc.)	75.7%	14.9%	4.4%	3.3%	1.7%
Pulses and oil seeds (e.g. peas, chickpeas, olives, sunflowers)	84.0%	11.0%	2.2%	1.1%	1.7%
Fruits	75.1%	14.9%	5.0%	1.7%	3.3%
Vegetables	65.7%	19.9%	9.9%	1.7%	2.8%
Meat and meat products	93.4%	2.8%	1.7%	1.7%	0.6%
Fish and seafood	91.2%	6.6%	1.1%	0.6%	0.6%
Milk and dairy products	81.8%	12.2%	3.3%	-	2.8%

Source: Authors' survey

As for the extent of food waste 58% of the respondents do not throw away food that is still consumable and about 17.7% throw less than 250 gr, finally 16.6% throw between 250 and 500 gr. As for the economic value of wasted food, it was revealed that for about 78.5% of the respondents the economic value of wasted food is less than 35 EGP (less than 5$), while for 14.9% of the respondents economic value of wasted food is between 42 and 140 EGP (6 - 20$).

It is evident that Egyptians' food purchases during Ramadan soar beyond all other monthly consumer averages, straining the efforts of ministers concerned with supply and domestic trade to keep up with demand. According to a recent study carried out by the National Centre for Social and Criminal Research (NCSCR), 83 percent of Egyptian families alter their food consumption habits during Ramadan in a way that augments their food bill for this month by 50 to 100 percent. If total annual consumer spending in Egypt comes to around LE 200 billion, LE 30 billion of this is spent in Ramadan, which is to say at a rate of LE 1 billion a day, the bulk of which goes to food in this month of "fasting". The NCSCR study observes that during this month

Egyptians spend 66.5% more on meat and poultry, 63% more on sweets, and 25% more on nuts and nibbles, and they host 23% more banquets and dinner parties. The study further notes that at least 60% of food on an average Egyptian family, and more than 75% of food in a banquet, goes to waste, which is to say tossed into the rubbish bin, during this month. According to statistics from the National Census Centre, in the first week of Ramadan Egyptians consume 2.3 billion loaves of bread, 10,000 tons of fuel, 40 million chickens, 200 percent more yoghurt and ghee, and some LE 9 million worth of dried fruits, which accounts for 35 percent of the annual trade in this festive staple (WikiIslam, 2015).

Willingness to behavioral change

This part deals with the notion of consumers’ willingness to change their behavior regarding food waste, thus to explore first the respondents’ perception of food waste reasons. It was evident from the results that most of respondents are familiar with such reasons, for instance 49.2% mentioned food is left in the fridge for too long time, followed by 43.1% of them that said food expired, and 40.3% indicated food has mold.

In addition respondents listed the following reasons: food does not have a good smell or taste, leftover, food does not look good and wrong preservation representing about 39.2%, 37.05%, 32.6%, 20.4% of the respondents, respectively. Packaging not being in proper size was at the end of the reasons representing about 6.1%.

Eventually, since respondents have clear vision about food waste causes or reasons, then could be willing to reduce such waste. Though, willingness is accompanied with information, so about 40.9% of the respondents mentioned that they will reduce food waste if the packaging was more suitable, followed by if they were better informed about the negative impacts of food waste on the environment, and better informed of the negative impacts of food waste on the economy, representing 38.7% and 37.0%, respectively.

In regard of respondents willingness to reduce food wastage, results indicated that 40.9% of the respondents have this will if the packaging of food was more suitable, 38.7% if better informed about the negative impacts of food waste on the environment, followed by 37.0% of the respondents who indicated they have the will if they were better informed of the negative impacts of food waste on the economy, 23.2% if labels were more clear and finally 21.0% of respondents mentioned their will is related to paying higher taxes on the basis of what they throw.

Finally, as for the information needed to reduce food waste, 53.0% of the respondents said that they need recipes with leftovers, 50.8% need tips on how to conserve food properly, 33.1% need organizations and initiatives that deal with food waste prevention and reduction, finally 28.2% need information on the freshness of products.

Conclusion

Reducing FLW is a multi-sectorial, multi-disciplinary and multi-factorial task. It requires networking and coordination between public institutions and private sector agencies in agro-industries, food quality and safety, NGOs, etc. Policies and regulations are key drivers for all actions aiming at reducing FLW but along with an effective participation and collaboration of all previous actors (FAO, 2014). Neither coordination and collaboration among relevant actors dealing with food losses and waste do exist, nor policies and regulations, but rather poor initiatives and scattered efforts.

The respondents showed particular characteristics which was related to their educational and income level. This requires to widen the scope of any coming studies to study different categories, and governorates either Upper or Lower or even Coastal areas to see the differences and variations when dealing with food waste. That will be through studying their behaviors by its three components; knowledge, attitude and trend from gender perspectives and then studying FLW that is the main cause of food insecurity in the developing countries in general and Egypt in particular. By this way food policies that deal with losses and waste will be formed on solid basis and upon current conditions and by the effective participation of all actors. Finally establishing the high council for food security that combines all relevant actors could be the key towards sustainable food systems in Egypt.

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THE IMPACT OF PRESCRIBED FIRE ON THE VEGETATION OF WADI EL-RAYAN WETLANDS (A PROTECTED AREA), WESTERN DESERT, EGYPT

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Abstract

The effect of man-made fires on the productivity of the key species *Phragmites australis* in Wadi El-Rayan wetland ecosystem was assessed. Three sites around the Upper and Lower Lakes were selected to monitor the fire effects during the period from 2005-2007. 2 sets of results were obtained showing the single effect of fire rather than the combined effect of fire followed by grazing in the same area. This study has been implemented during an accepted PhD study in the same area.

The rate of phytomass development for *P. australis* wetland in Wadi El-Rayan showed a continuous increase in the grazed area after fire rather than that in non-grazed area showed an increase followed by a decrease, however, the value of phytomass is much more in non-grazed area. The rate of phytomass accumulation in the lower lake showed a similar trend to that in the upper lake in non-grazed area. However, the absolute value of phytomass increase reached 56.7, 71.8 & 72.1 times more in the Upper Lake than those in the Lower Lake at winter, spring and summer seasons respectively.

A simple GIS time-based map comparison was provided to help understanding the time change of the vegetation figures of Wadi El-Rayan wetlands.

Keywords: *GIS, Phytomass, Vegetation, Prescribed Fire, wetlands, Wadi El-R*

Introduction

Wadi El-Rayan Protected Area WRPA declared by the prime-ministerial decree No. 943 in 1989 according to the law No. 102/1983 of the protected areas in Egypt and administered by the Nature Conservation Sector (NCS) of the Egyptian Environmental Affairs Agency (EEAA). WRPA is one of Egypt's 28 protected areas. The human interference in the protected areas is regulated by the environmental laws and regulating decrees. (Figure 1)

A well-developed reed beds had formed around WR Lakes (Upper and Lower) since the late seventies, with the *Phragmites australis* (Cav.) Trin. Ex Stud. As the dominant species invading the peripheries of Rayan Lakes (El-Hennawy, M. T., 2010). The Upper Rayan Lake shows more and well developed *P. australis* community due to the reasonable salinity level of the lake (around 1500 ppm), however, in the Lower Lake the salinity reaches 12000 ppm in some areas and up to 15000 in the extreme areas, which arrest the establishment and extent of the plant.

Firing in Wadi El-Rayan is a man-made practice feature affecting the wetland ecosystem, which is never previously studied. The cause of fires in Wadi El-Rayan is always a matter of guessing. It might be attributed to the production of new sprouts for un-authorized cattle and buffalo grazing in some locations around the Upper Lake by the local poor community inhabiting the villages located around the peripheries of the protected and wetland areas. Stopping the firing in the Lower Rayan Lake has strengthen the guessing that the cause of fire is for producing a palatable new sprouts for the grazing animals, that the wetland areas and vegetation development around it is extremely retarded the last years due to water decreasing level.

FIGURE 1: Map of Egypt Parks With the study site Wadi El-Rayan Highlighted in Red

Studying the fire effects on the wetlands led to several facts and reports. The use of fire in coastal marsh management is described as a means to remove dead vegetation, re-establish lower succession stages, or return the marsh to an early hydric community. Fire prevents accumulation of organic matter and thus impedes elevation of the marsh and succession to upland communities. (Diaber, 1974). The literature indicates no adverse effects of fire on fish or aquatic invertebrates. Ruffed grouse, sharp-tailed grouse, spruce grouse, and ptarmigans obtain new habitat from fire. Habitat changes following fire benefit some large mammals but neither harm or benefit most small mammals. (Kelsall, *et al*, 1977).

Disturbance related to chance perturbations, water depth, and the incidence of fire accounted for much of the variation in the sedge meadow community. Annual burning (presumably by local residents) maintains the meadows against invasion by shrubs, increases nutrient mineralization, and provides a pronounced change in albedo which permits earlier spring growth. (Auclair, *et al*, 1973)

Immediate and long-term effects of fire on wildlife are reviewed. Included are discussions of changes in species composition and energy flow following fire, changes in density and overall abundance of wildlife following fire, and various case histories to support the major points presented. The evolution of birds and mammals in burnable habitat (including the effects of fire upon wildlife speciation as a result of fire) and adaptation of birds and mammals to flammable habitat are examined. With reference to wetlands, burning results in open water and encourages seed-bearing plants which are valuable waterfowl foods. (Bendell, 1974).

Today, the same conditions are still there especially in the Upper Lake and reflected on the greater area and density around it rather than around the Lower Lake. Common reed germination may be decreased at salinity levels greater than 5000 ppm (Briea, 2006). This is very clear in the Lower Rayan Lake, when the water level decreased after 1999 and the shoreline of the lake greatly retarded, the soil has dried up and the existed vegetation has died (except the woody species such

as *Tamarix*). The reed stolons run towards the new shoreline for long distances trying to reach the moist, but the visible growth rate and vitality and vigor are so weak due to the high salinity of the lake in these areas which reaches more than 12000 ppm. It is easy to notice the stolons and new seedlings of *Phragmites* trying to establish themselves in these areas (El-Hennawy, M. T., 2010). The study of fire effect on the phytomass of the *Phragmites australis* on Wadi El-Rayan wetlands will be the matter of this study.

Materials and methods

Three sites were selected to study the accidental fires in the wetlands of WRPA. GPS coordinates for the location were recorded immediately after fire. Two sites were located around the Upper Lake and one site located at the south of the Lower Lake. One of those sites was permitted to cattle grazing after fire around the Upper Lake. The fire sites were visited by three months intervals after the firing time. In each site, an average of two-randomly selected clip quadrate (1.00 m^2) was used for phytomass assessment (Sarvis, 1923 and Weaver & Clements, 1957). The above ground vegetative parts were clipped, cleaned and sorted out. Fresh samples were dried at 100-110 ^oC for constant weight. The productivity figures presented in this study are based on dry weight basis. The whole site was detected for vegetation and lakes area change through 2005 to 2015. All image processing has been conducted using Intergraph ERDAS Imagine v. 2013 and ESRI ArcGIS v. 10.2 Software.

Results and discussion

A. The Effect of Fire on The Rate of Phytomass Accumulation

1. The Upper Lake

The rate of phytomass accumulation increased by 209.3% from winter to spring, and decreased by 31% from spring to summer. Figure (2) showed the variation of phytomass after fire took place in autumn 2005 in the Upper Lake.

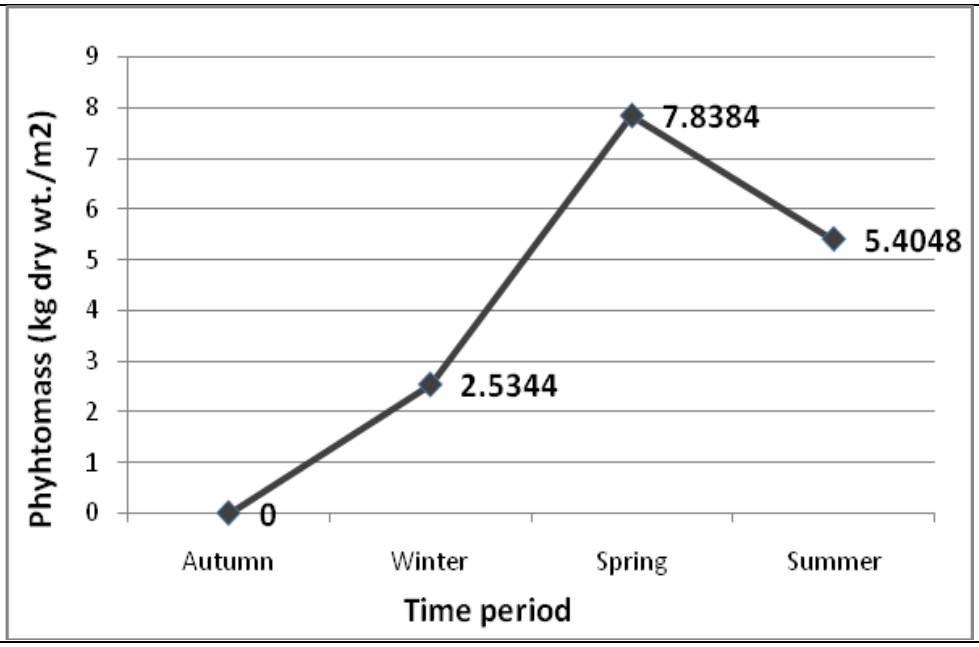

FIGURE 2. Variation of phytomass after fire in the Upper Lake area (Autumn 2005)

2. The Lower Lake

The rate of phytomass accumulation increased by 144.3% from winter to spring, and decreased by 31% from spring to summer. Figure (3) showed the variation of phytomass after fire took place in autumn 2005 in the Lower Lake

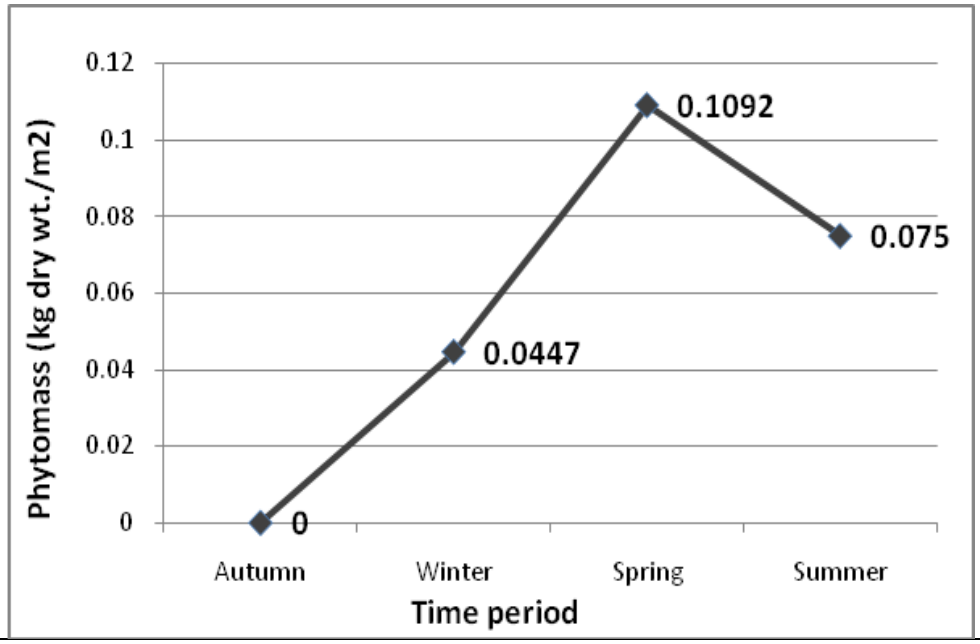

FIGURE 3. Variation of phytomass after fire in the Lower Lake area (Autumn 2005)

As a conclusion, the same trend was realized for the phytomass accumulation rate in both lakes of Wadi El-Rayan, after firing in a three-month interval, which is an increase followed by a decline in summer season.

However, the absolute values of the phytomass varied widely from the Upper and Lower Lakes sites after firing. It was found that the absolute values of phytomass were significantly higher in the Upper Lake compared to the Lower Lake (Figures 2, 3 and 4) in the period of the study.

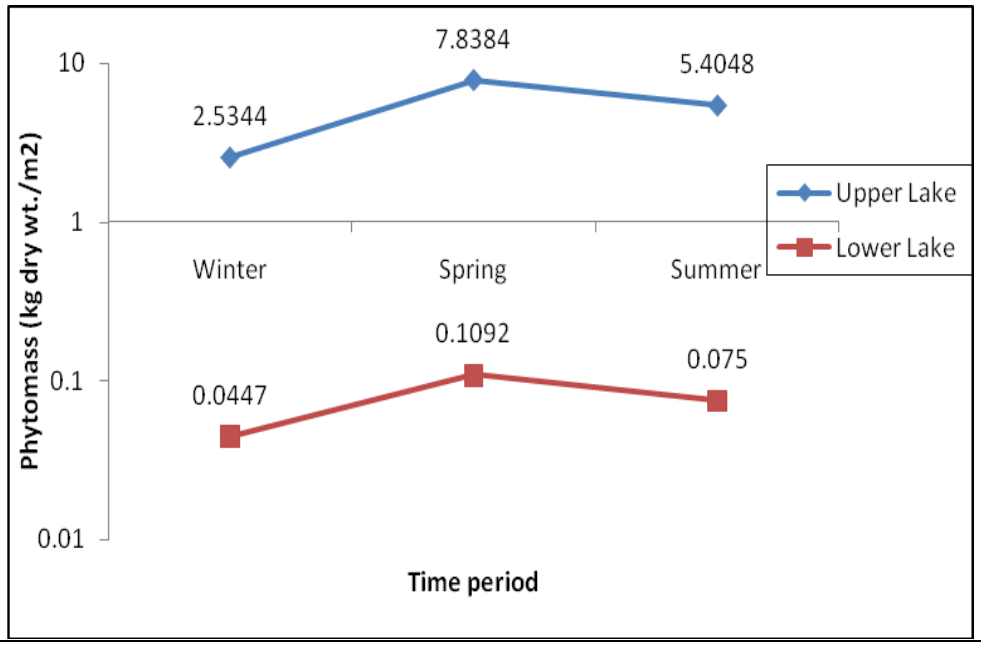

FIGURE 4. variation in the absolute values of phytomass in Upper and Lower Lakes after firing

B. The Combined Effect of Fire & Grazing on The Rate of Phytomass Accumulation
The rate of phytomass accumulation was assessed in the Upper Lake area after occasional fire followed by cattle grazing. The rate of phytomass accumulation was continuously increased by 147.9% from winter to spring, and 56% from spring to summer. Figure (5) showed the variation of phytomass after fire took place in autumn 2005 in the Upper Lake.

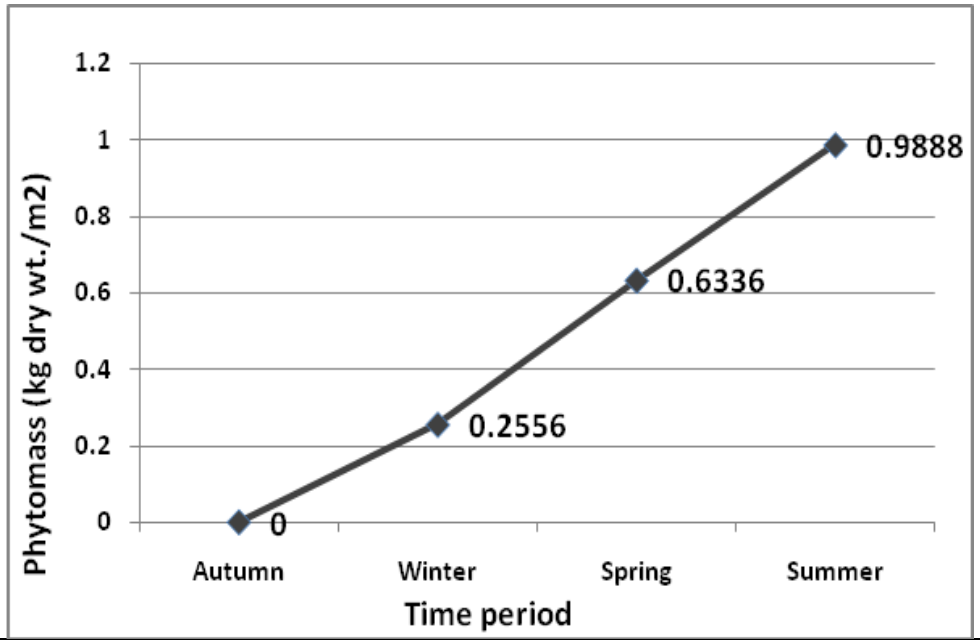

FIGURE 5. Variation of phytomass after fire & grazing in the Upper Lake area (Autumn 2005)

C. The change detection in wadi el-rayan protected area
A positive relationship between the water body of the lake area and the area of vegetation cover was shown by Figure (6). The area of vegetation cover changed from 973.42 acre in 2005 to 1324.58 acre in 2015, along with a tiny increase in the water body area of the Upper lake with only 134.5 acres and mostly occurred in the Upper lake.

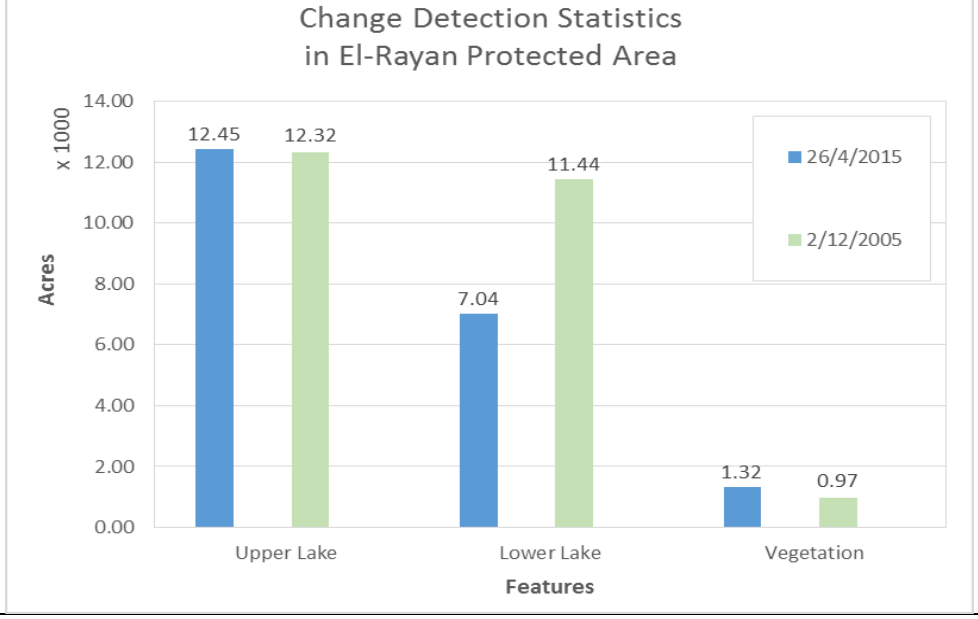

FIGURE 6: The change detection from 2000 to 2015

	Area (Acres)	
	26/4/2015	**2/12/2005**
Upper Lake	12453.90	12319.40
Lower Lake	7038.96	11439.40
Vegetation	1324.58	973.42

Figure 6 also shows remarkable decrease of the water body of the Lower lake area and the area of vegetation cover. It decreased from 11439.40 acre in 2005 to 7038.96 acre in 2015 with a noticeable decrease in the vegetation cover.

Figure 7 shows the change in vegetation and water areas of the Upper and Lower lakes of Wadi El-Rayan from 2005 to 2015.

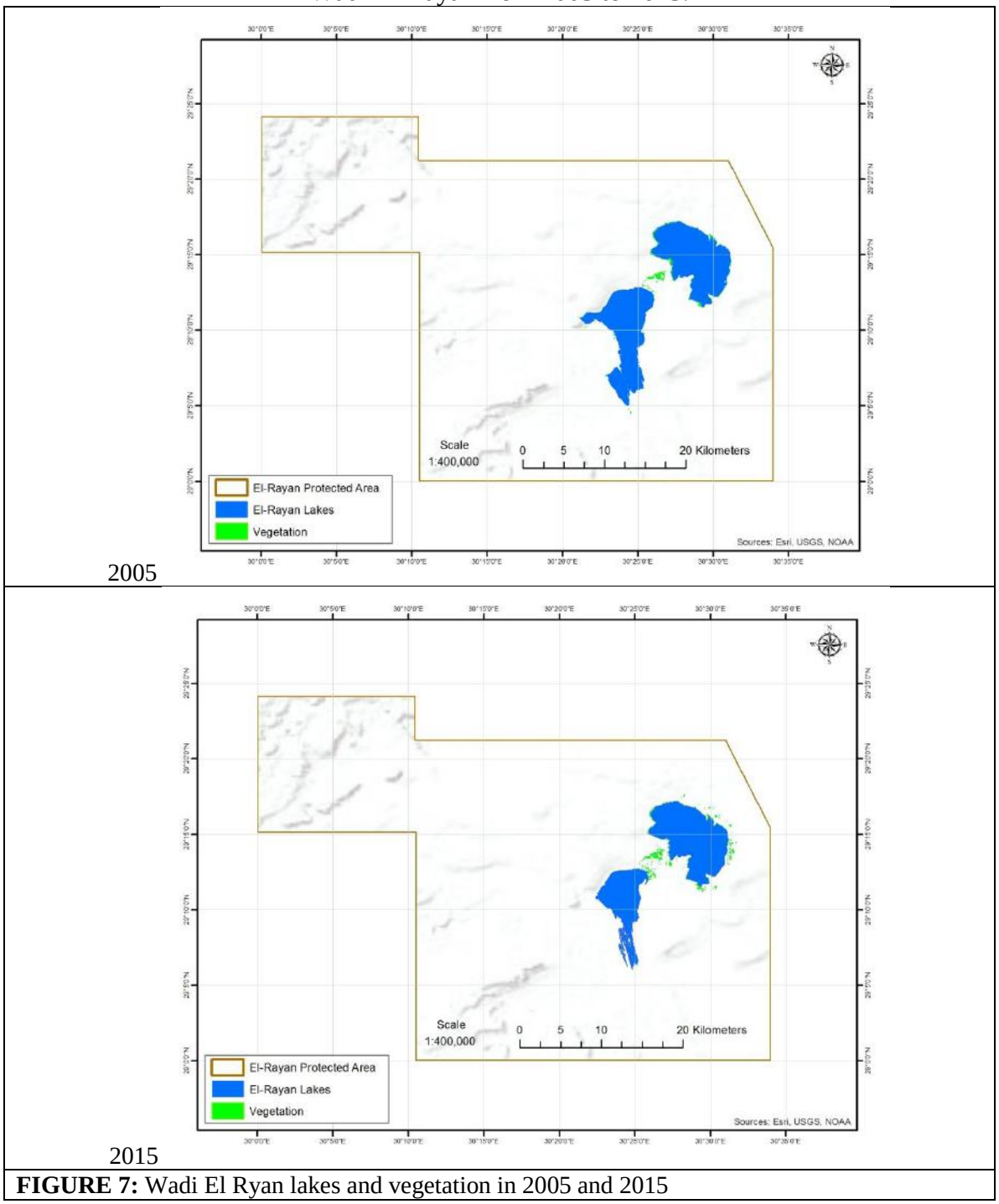

FIGURE 7: Wadi El Ryan lakes and vegetation in 2005 and 2015

Conclusion

The monitored fires in this study were dated September 2005-2007 on the wetland cover around both of the Rayan lakes. The rate of phytomass development for *P. australis* wetland in Wadi El-Rayan showed a continuous increase in the grazed area after fire, while that in non-grazed area showed an increase followed by a decrease, however, the value of phytomass is much more in non-grazed area.

The rate of phytomass accumulation in the lower lake showed a similar trend to that in the upper lake in non-grazed area. However, the absolute value of phytomass increase reached 56.7, 71.8 & 72.1 times more in the Upper Lake than those in the Lower Lake at winter, spring and summer seasons respectively.

Common reed sprouts rapidly from surviving rhizomes after fire. Sprouts may appear as soon as 5 days after fire (Ward, 1968). This is almost the case in Wadi El-Rayan Upper Lake. Fires that took place in the Rayan Lower Lake may retard its vegetation sprouts up to more than one month. Rarely is common reed abundance decreased by fire, and post fire recovery is typically rapid. In the case of Wadi El-Rayan, a huge stock of the common reed rhizomes existed especially in the area of the Upper Lake and the connecting channel.

Common reed post fire abundance (cover, biomass production, and/or density) is rarely different from pre-fire abundance by the 2nd or 3rd post-fire year (Ward, 1968; Ailes, 1993). It is not uncommon for burned sites to have greater common reed abundance than unburned sites (Greenall, 1995; Thompson & Shay 1985).

For the case of Wadi El-Rayan, the same trend was realized for the phytomass accumulation rate in both of Wadi El-Rayan lakes, after firing in a three-month interval, which is increasing followed by decreasing rate.

In Wadi El-Rayan case, it is not urgently needed as the sedge meadows are still there. The edaphic factors are also supporting the same composition as the soil particle analysis shows frequent silt and clay particles than sand; the latter is supporting the shrubby and woody stage such as *Tamarix nilotic*a growing frequently on the external borders of the *Phragmites australis* wetland especially in the lower Rayan Lake with frequent sandy soils. So, even there are no recorded investigations about the effects of fire on the wetland productivity in this area, it is not recommended to allow firing processes.

The study of the fire impact on the productivity of Wadi El-Rayan wetland was very helpful in providing suitable management actions to conserve the integrity of the ecosystem.

Fires in common reed marshes can be used to benefit wildlife, but can also negatively impact nesting birds. Prescribed fires should avoid destroying currently used nesting habitat.

Grazing must not be allowed in case of weak management system which can not limit and monitor it within the proposed boundaries or seasons.

The produced GIS maps were very helpful to show the picture of the identified high/low wetland vegetation productivity sites in addition to the potential grazing sites among these sites taking in consideration the seasons and sites of actions.

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BIOCHAR FROM MUNICIPAL WOOD: A KEY STRATEGY TO PRODUCE ADDED VALUE PRODUCTS FROM WASTES

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Abstract

Solid woody wastes collected from pruning of municipal trees is a major source for producing energy and added valued materials in countries, though millions of tons of such biomass are feasible. In this study, the pyrolysis of wood collected from pruning of plane tree at different temperatures and different sizes has been investigated. Pyrolysis process was performed in a fixed bed batch reactor under adiabatic conditions. The ultimate goal was the production of maximum ratio of biochar mass from wood waste. Humidity, temperature, durability and the effect of size in 400, 500, 600, 700° C were tested in triplicate. The particles in 4 sizes were prepared. All parameters were weighed before and after pyrolysis to obtain the biochar mass fraction. According to the results obtained from the pyrolysis process, the best temperature to yield desirable output qualitative attributes was obtained to be 600°C and the most optimal resistance time was measured to be 10 minutes in which there would be sufficient time for gaseous carbon to reproduce as the Biochar. In term of the size of the components, the cubes with the 1 cm^3 in size and weight of 0.6 g/cm^3, need less energy to convert to Biochar and carbon and give high quality Biochar production as a final product.

Kewords: *Pyrolysis, Biochar, Activated Carbon, Solid Woody Waste*

Introduction

The use of alternative -energy from biomass has increased recently and this is due to the sharp decline in oil reserves and rising demand of energy in the world,which has gotten theattention of politicians from different countries considering the cost of energy production (Demirbas, 2001; Yang et al., 2010).Biomass:materials and products are often used as fuel such as trees, fast-growing grass, agricultural residues,waste materials such as straw, sugarcane fiber, rice husk, forestry and forest residues,waste of wood construction and industry (Crocker et al., 2006; Medlock et al., 2001).It also contains a small amount of nitrogen, sulfur and ash compared to conventional fossil fuels,which if burned, greenhouse gases including NO_x, SO_2,and soot is less emitted compared to current fossil fuels (Zhang et al., 2006; Borjesso et al., 1996).

Synthetic gas which is the product of biomass pyrolysis can be used in combination with other elements to generate heat and power in the industry. Part of the emitted synthetic gases are H_2, CO and CO_2, that when converted to synthetic fuel, can be beneficial to the environment or can be selectively used to convert hydrocarbon with high molecular weight to catalyst.

In order to convert the fast-growing grass to produce bio- oil in the process of pyrolysis, a great deal of research on the pyrolysis reactor design, pyrolysis conditions, product characteristics, and final quality improvement was done. However, very little attention has been paid for the detailed study of pyrolysis process and the results of the pyrolysis products at different temperatures. In future studies, the chemical composition and physical behavior of bio - oil can be determined better by specifying the pyrolysis process and its products.

Damirbas, 2004 has studied biochar production from agricultural residues such as the corncob, olive husk, and teawaste. In this research,the temperature range of 670 °C to 1070 °C and particle size of 0.5 to 2.2 mm have been considered in order to produce biochar.

The process of biochar production as a whole is divided into three stages (Damirbas 2004):

1 Biomass →Water + Unreacted Residues
2 Unreacted Residues → $(Volatiles + Gases)_1 + (Char)_1$
3 $(Char)_1$→ $(Volatiles + Gases)_2 + (Char)_2$

In the first stage, only some moisture and volatiles are gone; in the second stage, the initial biochar is achieved; and in the third stage which is done at high speed, pure carbon formation and chemical changes occur and carbon is left in Biochar. According to the chemical equations below, maximum thermo chemical reaction has taken place in the pyrolysis process:

4 $C + 2H_2 \leftrightarrows CH_4$
5 $C + O_2 \rightarrow CO_2$ and $C + CO_2 \leftrightarrows 2CO$
6 $C + CO_2 + H_2O \leftrightarrows CO + H_2$

In the chemical formula 6, the emitted gas from the process can be used as gaseous fuel.
The output Biocharfrom the process can be calculated in accordance with the following formula (Nunes et al., 1996):

$$Biochar\ yield = \frac{\left(\frac{A_b}{A_c}\right) - \left(\frac{A_b}{100}\right)}{1 - \left(\frac{A_b}{100}\right)} \quad 7$$

Where b is the weight percentage of dry biomass and c is the percentage weight of dry Biochar. The study of agricultural residues such as rice straw, straw and corncob in the temperature range of 300 to 700 °C was conducted by Chen et al. 2008 including the investigation and drawing of diagrams showing the amount of lost material by wood waste. With an increase in temperature, the amount of lost material increases and the amount of Biochar which is left decreases.
Samples temperature has been increased from the room temperature up to 800 °C with the temperature rate of 20 °C /min with the flow rate of nitrogen and a mixture of nitrogen and oxygen by 6%, 14 % and 21% of oxygen. Lost materials in pyrolysis process have been studied and analyzed using TG and DTG curves (Chen et al., 2008).

Material and methods

In this experiment, a pyrolysis reactor (single batch, fixed bed) and thermal conductivity measuring device of wood were used. Samples from plantain cut in the spring of 2014 were used. Samples were prepared in four different sizes of 0.01 mm^3- chips - 50 mm^3, 20 * 5 * 0/5 mm -cubes with a volume of 1 cm^3 and one 1 in^3 - 15 /625 cm^3 which have been shown in Figure 1 to 4, respectively. A piece with the format of 10*2.5*1 cm was prepared from the same tree, measuring thermal conductive of samples. Humidity changes this quantity.
Standard ASTM E871 was used to calculate the moisture content of wood and for this purpose the wood was put in the oven at a temperature of 103 °C for 16 hours and the humidity was measured and calculated according to this standard. Firstly, all the initial weight values were measured using an accurate digital scale with three decimal digits, Shinko, Japan, and secondly, moisture content was measured and the weight was recorded accurately in accordance with standard ASTM E871. Samples were located in the reactor subsequently.
Heat rate was applied at 40 °C/min.the sample was placed in crucibles inside of the reactor. Thermocouples were embedded in the crucibles and the resistance timewas calculatedby forth sensor after reaching the desired temperature. A relative vacuum was provided by a vacuum pump to maintain the oxygen level of the process medium. After leaving samples in a

vacuumed glassy environment, they werechilled at room temperature and then weighed with the digital scale.

Results and discussion

The results in Table1 show the percentage of biochar production at different temperatures with 5-minute residence time, in the specific dimensions.

Table1. Effect of temperature and Biochar size on the production yield (%) at the resistance time of 5 minutes

(C°) Temperature	400	500	600	700
0.01 mm^3	44.2344	37.5325	22.4338	21.4225
50 mm^3	39.8262	27.5821	23.7686	23.7081
1 cm^3	32.8392	28.5213	23.8267	27.3194
15.625 cm^3	29.3371	24.3799	22.4069	21.6264

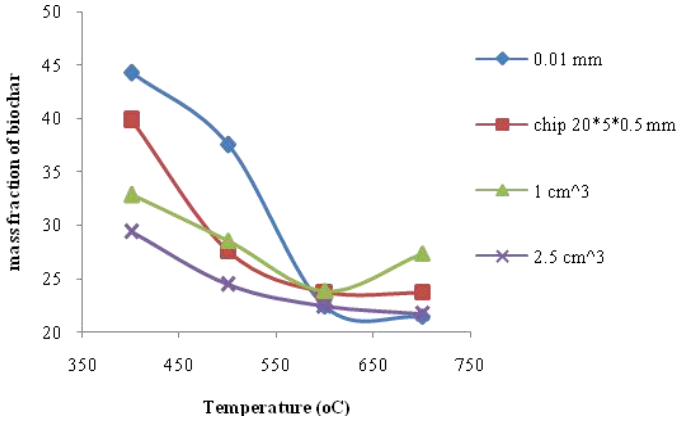

Figure 1. Variation of mass fraction of biochar with temperature and size (within a fixed time of 5 minutes)

Figure 1 shows the percentage of biochar production to temperature within a fixed time of 5 minutes, at temperatures of 400, 500, 600 and 700 °C. The residence time of the product in the reactor is calculated when the internal temperature of the reactor reaches the desired temperature. This chart has been prepared for four different sizes of the wood from plantain tree, which includes cartridge with the average size of 0.01 mm^3, Chip with the average size of 50 mm^3, and two other samples with similar cubes, including the weight of 700 mg per cm^2 which is equal to a cube with sides of 1 cm and a cube with sides of 2.5 cm.

According to the Figure 1, it can be concluded that with increasing temperature over a period of specified time at high temperature, the rate of biochar production decreases.The decline of biochar production could be attributed to a rise in temperature and along with it, an increase in gas production.Since the amount of generated heat increases, there is not enough time for a certain amount of gas with carbon to return on produced biochar, to react with it.

At higher temperatures due to the proximity to the gasification step,a large amount of the product is converted to gas, hence sublimation process occurs in which the majority of carbon at high temperatures reacts with hydrogen and oxygen, which have been decomposed from water vapor, and is emitted in the form of methane and carbon dioxide.However, at low temperatures,the reaction time is larger and the energy of carbon molecules is less, thus the production of biochar increases.

The percentage of biocharproduction decreases with temperature and in its smallest size, according to the Figure1, it becomes approximately half the original sizefrom 500 to 600 °C.

With the experimental particle sizes getting larger, the percentage of biochar production decreases slightly.In the case of the cubes of 1cm^3, biochar productiondecreases when the temperature increases from 400 to 600 °C.
For cubes with 1cm length for each side, biochar production reduces with temperature in the range of400 to 600 °C. In the contrary, the biochar production increased for the 700 °C which is due to the return of a significant amount of fumes that contain carbon on biochar. For smaller considered dimension of wood pieces, biochar production decreases with temperature. The reason can be attributed to the small size of particles and their high level of exposure to heat. Consequently, the outer layers of small pieces turn into gas quickly by increasing the temperature which reduces the amount of biochar production. Damirbas 2004 stated that the purity of carbon in biochar and the amount of the emitted gases increases with temperature. Chen et al., 2008, calculated the lost material in the pyrolysis process. The results released that the amount of removed material increases with temperature.
Iman, 2012 announced that increasing in the moisture content, caused the less biochar production and more released gases. Park et al., in 2012 reported that with an increase in temperature, the amount of gas and oil increases and biocharproduction decreases.
At 600 °C and a low heat rate, the amount of lost material is more than other cases and other temperatures.

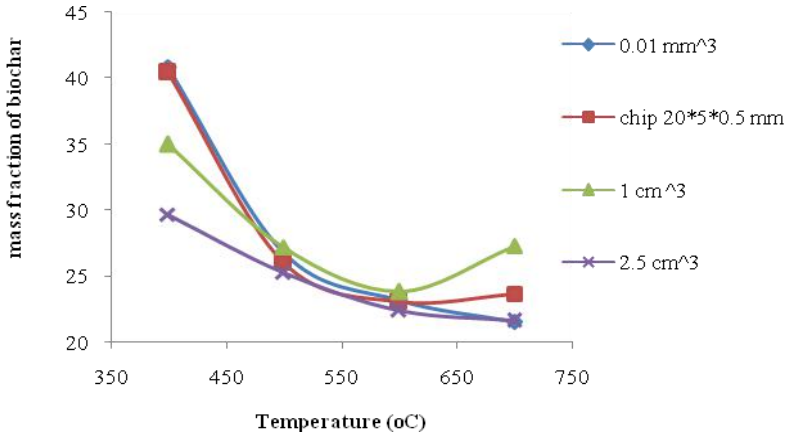

Figure 2.Variation of mass fraction of biochar with temperature and size (overthe fixed period of 10 minutes)

Figure 2shows the amount of biochar production to the temperature over the fixed period of 10 minutes at the temperatures of 400,500, 600 and 700 °C and the fact that in this case, the amount of biochar production is more than theprevious experiments (Fig. 2).Because in this case carbon has more time to react withbiochar and turn into biochar; here, with an increase in the temperature, the amount of biochar which is left will decrease as well.

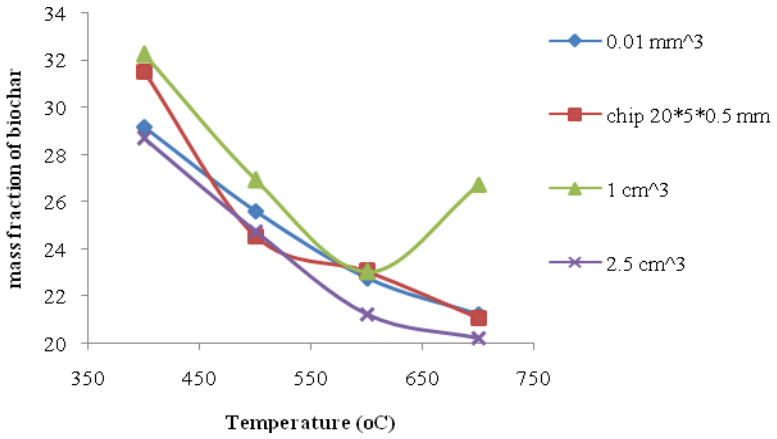

Figure 3.Variation of mass fraction of biochar with temperature and size (over the fixedperiod of 15 minutes)

Figure3 shows the percentage of biochar production in relation with the temperature over the fixed period of 15 minutes at temperatures of 400,500, 600 and 700 °C. In this case, the amount and percentageof biochar is less compared to the previous experiments.In the third experiment, since the most optimal time is given to the carbon,the carbon which is on biochar reacts with water vapor molecules in specified heatagain, and higher levels of carbon molecules are emitted from the reactor in the form of carbon dioxide and methanegas.In this instance with an increase in the temperature the amount of biochar which is left will decrease. (If is possible put one or two citation of similar results or investigation as in experiment 2 and 3)

Conclusion

Every year municipal waste from pruning trees is considered as a major source for producing energy and value-added materials in the countries with no administrative approach in this regard in Iran. In this research, the pyrolysis of wood waste and urban pruning of plantain at different temperatures and in different sizes have been studied. According to the results obtained from pyrolysis process and the comparison of the parameters with each other the best and recommendedtemperature was 500-600 °C and the optimal resistance time was in the maximum temperature of 10 minutes. At this temperature there is more opportunity for carbon to return to biochar.Moreover,in terms of the size of the pieces, cubes of 1 $cm^3$and weight of 0.6 g/cm^3 need less energy to convert tobiochar and active carbonand also they have high quality.

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Review paper
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NEGATIVE FEEDBACKS DUE TO PRESENCE OF HEAVY METALS AND AGRICULTURAL TOXICANTS IN THE ENVIRONMENT: A SOIL QUALITY PERSPECTIVE

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Abstract

Nowadays, environmental pollution is a crucial issue that humans are facing repeatedly. Population growth, urban development, industrial expansion, and irregular/excessive interventions of human in nature have all contributed to the rapid environmental degradation. Most of the important soil contaminants are heavy metals, mainly resulting from human activities like mining, industrial waste materials, vehicle emissions, herbicide and detergent application, fuel/energy manufacture, urban wastes, fertilizers, paints, etc. Mercury, arsenic, cadmium, lead and chromium are the most prevalent pollutants responsible for environmental pollution, in particular, rhizosphere zone and adversely affect the health of the ecosystem. Chemical substances, hazardous waste materials, domestic waste and sewage directly and/or indirectly impact soil texture and structure in a way that at later stages mitigate the decomposition activity of soil microorganisms and eventually make the soil infertile. Recently, several studies have been conducted across the globe over possible remediation approaches to successfully reduce the soil contaminations (SC). This review strives to reemphasize the SC issue, its effect on ecosystem health, efficient methods leading to removal of the heavy metals of contaminated soils, and rehabilitation and retrieval of soil resources in such a way that the soil can be used for healthily agricultural purposes.

Keywords: *Soil resources, environment, heavy metals, pollutants, agriculture*

Introduction

The soil is considered as one of the natural purifiers; and in addition to supplying nutrients to living organisms, it has purifying properties. This feature is mainly achieved by physical (water permeability within pores), chemical (absorption and evaporation) and biological (decomposition and decay of organic matter) attributes (Feizy, 2010). 95% of human food is achieved by earth (Khaksarian, 2010). Therefore, planning for possessing a producing and healthy soil is essential for human survival. Entering materials, biological organisms or energy into the soil trigger its quality alteration and deform its natural status (Rahmani, 2003). Unfortunately, soil now gets polluted due to anthropogenic activities. Traditionally, soil, air and water have been traditionally utilized as hosts for disposal of wastes. The nature of these waste products can be divided into four major categories: agricultural, municipal, industrial and nuclear waste materials (Razavi Tosi, 2005). Some of them are presented in the Figure 1.

Air pollution caused by automobile fumes can also trigger soil contamination and have a negative effect on local air quality. Pouring such toxic materials as solvents and detergents can also extend soil contamination (Kordavani, 2010). As one of the important components of the environment, soil is the most significant recipient of industrial and agricultural wastes. Upon entering the soil, these materials become part of a cycle that will impact soil biological, geological and chemical processes in a number of ways. Thus, information on the nature of these pollutants as factors contributing to ecosystem disorder and their chemical behavior

within the soil is mandatory for imposing scientific management for cleansing and efficiently neutralizing them.

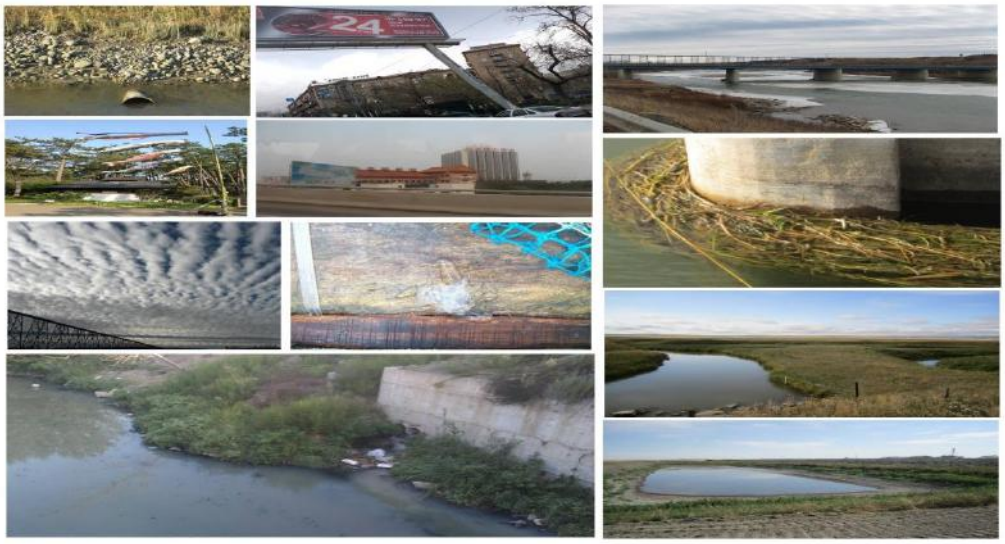

Fig.1. The composite image show polluted water discharge in Canada; city based air pollution in Armenia; wilderness area in Japan, garbage center in Canada; industrial pollution and water borne sewage and debris in the US, air pollution in Canada, polluted channel and disposal of waste water into a local river (Zarjoub River) Rasht, Iran; and polluted industrial waste water storage in North America and India (Photo courtesy: Saikat Kumar Basu & Peiman Zandi).

Heavy metals are natural constituents of the earth's crust. At a lower concentration they appear to be less harmful and can even act as trace elements useful for living organisms. However, these metals are very toxic once they appear in their stable oxidation states. They react with the bio-molecules of the living beings to constitute stable biotoxic compounds (Duruibe et al., 2007). In comparison to plastic and petroleum (organic contaminants), they linger more persistently in the nature. Depending upon the soil pH and speciation they can be mobile and either transfer to an aquifer or become available to living organisms easily. Their toxic properties may result in drinking water contamination, contaminated food chain and facilitate higher concentrations of air pollutants near the emission sites (Hashim et al., 2011).
Overall, the remediation process in contaminated soil and groundwater can be conducted through the following: wholly destruction of pollutants, extraction of contaminants out of disposal wastewaters, keeping the pollutants in the forms of stabilizing (non mobile) and less toxic, elicitation of non-contaminated materials and their relevant recycling of contaminated substances, and to avoid further expansion of polluted materials to other environments (Hashim et al., 2011).

Industrial pollutants and heavy metal accumulation in soil

The elements present in sewages are known as special elements and the main reason of such nomination refers to the reality that they are rarely found in common soils except in agricultural soils that are irrigated by municipal waste waters (Mirsal, 2013). These elements, in brief, are: arsenic, cadmium, cobalt, chromium, copper, mercury, molybdenum, nickel, selenium, vanadium, zinc, iron, aluminum and lead (Hopenhayn-Rich et al., 1996). These heavy metals are very important, even at low levels of concentration because of being unable to be decomposed and also their physiological adverse effects. Some of these adverse effects comprise disruption in soil biological activities, toxic effects on plants and adverse effects on human health because of the influx of substances in the food chain (Davies, 1995).

Arsenic (As): This element is a devastating environmental pollutant, is mostly employed in dyeing, tanning, and textile industries; and can be found easily in the laundry powders and detergents. With reference to having terrific toxicity, it is now widely applied in the production of fungicides, insecticides and pest expulsion poisons. Similar to phosphorus conduction in the soil, it is also hardly mobilized and/or removed from topsoil area. The permissible limit for 'Ar' in the edible products has defined 2.6. The municipal sewage seems to be the main trajectory agent responsible for soil contamination (Watt et al.,1993).
Arsenic (Sodium Arsenite) is a white metalloid solid element widely distributed in the nature and present at an average concentration of 2 mg/kg. To form organic and inorganic compounds, arsenic can combine with other elements. Compared to organic arsenic compounds, inorganic arsenic compounds are thought to be even more toxic and detrimental for health. The researchers have found that arsenite is more toxic than arsenate (Ahmed et al., 2008; Hashim et al., 2011). Arsenic occurs in crystalline, powder, amorphous or vitreous forms in all rock, soil, water and air. Industrial processes such as smelting, mining, and coal-fired power plants, agricultural pesticides (22% of the world production of arsenic) and chemicals for timber preservation (70% of the world production of arsenic), all contribute to the presence of arsenic in air, water and soil (Bodek et al., 1988). Arsenic results in the inhibition of growth, photosynthesis and reproduction in plants and even lethality depending on the species and time of exposure. Arsenic is also associated with skin damage, kerratosis and skin cancers and problems of the circulatory system (Scragg, 2006).
Cadmium (Cd): Cadmium is a heavy metal that is one of the greatest concerns in the environment, because of its toxicity to animals and humans. Plants can easily uptake cadmium and transfer it to other organs (Campbell, 2006). Cadmium has a chemical similarity to Zn, an essential micronutrient for plants and animals. Its substitution with Zn may cause the spoiling of metabolic processes in plants (Campbell, 2006). Effects on birds and mammals are mainly due to kidney damage (Weggler et al., 2004). Once it is in the environment, it can remain present for a long time. It can be transported from one location to another, in particle forms that are either blown by the wind or washed away by water. The most important symptoms of cadmium toxic effects on plants are stunning and chlorosis (McLaughlin et al., 2000). It is well consumed in the paint and plastic manufacturing industries. Non contaminated soils usually contain less than 1 ppm Cd, but in the soils around the factories the mentioned amount has reported to be near 1700 ppm (Jackson and Alloway,1992; Watt et al.,1993).
Cobalt (Co): Cobalt is a hard, brittle metal that occurs naturally in the environment and is a common by-product of nickel and copper mining activities (OMEE, 1993). Burning coal or oil and use of cobalt containing chemicals can enter this metal to the environment. It can be accumulated in plants (very small amounts of cobalt) when they are grown in the contaminated soil. Cobalt cannot be destroyed once it has entered the environment; however, it may change forms by combining with other chemicals (Chaney, 1982). Cobalt is used greatly in the production process of different alloys, colors, glazes, coatings and writing inks, usually it does not exceed 10 ppm in the soil. Studies show that Co often adsorbed on clay colloids, and only has transaction with other heavy metals like Cu and Zn. It generally has low concentration in the soil and sewage as well as factories waste waters (Razavi Tosi, 2005; Kordavani, 2010). The element has not yet caused or reported to have any adverse complications in the available literature.
Lead (Pb):The amount of lead concentrated in the environment is basically originated from natural and human (anthropogenic) activities. Despite having recession status in the market, its secondary production is increasing due to its non-ferrous, recycled nature. It is also used as an additive factor to improve gasoline performance. Some of the plant anti-pest toxins are slightly composed of the element (Kordavani, 2010). Unfortunately, the so-called heavy metal first pollutes the air, and then transfers into the soil with the aid of rain. The allowance level

determined for ‘Pb’ consumption in human solid feeds should not overlap 600 µg per day due to its nature of bioaccumulation. According to the daily reported records, humans take in 10 to 100 µg Pb through inhalation, whilst the range goes upper than 300 µg a day via nutrition pathway (Jackson and Alloway,1992; Alloway,1995a).

Aluminum(Al): Aluminum toxicity is one of the greatest limitations to agricultural production in acid soils. It is estimated that approximately 50% of the cultivable soils in the world possess high acidity, principally in the tropical regions (Venturini-Soriano and Berthon, 2001). Despite advances in the management of Cerrado soils, the correction of soil acidity to neutralize the toxic Aluminium in layers 20 cm below the surface is costly and difficult to operationalize. Thus, even in well-managed soils, subsurface layers might have a high acidity and high degree of saturation with toxic forms of Al, limiting root development in a cultivar sensitive to this element (Sánchez and Salinas,1981). Aluminium causes rapid inhibition of root growth (Kochian et al. 2004). Aluminium sensitive cultivars have poorly developed root system and are unable to explore the deeper layers of the soil, limiting water and nutrient uptake. As a result, cultivars sensitive to Aluminium toxicity are also susceptible to water stress (Taylor,1988).

Copper(Cu): Copper is a transition metal which is the third most used metals in the world (VCI, 2011). It is essential for the growth of both plants and animals. In plants, Cu is especially important in seed production, disease resistance, and regulation of water. In the soil, Cu strongly complexes to the organic and will be found in solution as ionic copper, Cu (II). At pH 5.5, the solubility of Cu is dramatically increased (VCI, 2011). Copper is essential for the plants and animals, but in high doses it can cause adverse effects on the plants and humans. Directly, it has negative effects on crop growth and yield, and indirectly, it slightly enters into the human food chain or bio-accumulates with a potentially negative impact on human health (Martínez and Motto, 2000). Fortunately, despite its complex interaction with the environment most copper introduced into the ecosystem rapidly converts to an innocuous and stable form.

Mercury(Hg): Mercury is one of the most hazardous environment pollutant metals (harmful to human and aquatic creatures) partially enters the soil or surface water by direct disposal of electric lamps, explosives, and wastewaters made by factories and application of fertilizers (Alloway,1995a). Mercury is greatly released into the air by human activities like mining, smelting, fossil fuel and solid waste combustion. Naturally, it enters the soil through normal degradation of soil, rocks and minerals through weathering. The toxic form of ‘Hg’ in the marine environment is known as Monoethylmercury which is usually travel via marine food chains and later on underlies serious damage to humans.

Nickel(Ni): Nickel is a transition element that occurs in the environment only at very low levels, and does not accumulate in the living organisms. It's essential in small doses, but once the maximum bearable amounts are exceeded, it can be dangerous. The animals that live near refineries have various kinds of cancer due to the nickel toxicity. The major sources of nickel contamination in the soil are fossil fuels and nickel mining (Khodadoust et al., 2004). In acidic soils, Ni becomes more mobile and often leaches down to the adjacent groundwater and as a part of wastewater streams can also end up in surface water. Microorganisms can also suffer from nickel accumulation. Plants or animals don't accumulate nickel, so it can enter into the food chain (Pourbaix, 1974).

Zinc(Zn): Zinc is another transition metal which occurs naturally in soil, water and air, but its concentrations are rising unnaturally, due to the man-made additions. Industrial activities such as waste combustion, steel processing and mining add most of Zn to the soil. The acidity of waters can be increased by zinc (Davies and Jones,1988). The groundwater can be contaminated by water-soluble zinc that is located in soils. Plants often can uptake Zn, but they can't handle additional zinc due to the accumulation of Zn in soils. Finally, Zn can

interrupt the activity of microorganisms and earthworms in the soil, thus retarding the breakdown of organic matter and disrupt plant activities (Greany, 2005).

Chromium(Cr): Chromium has an antifouling feature and is widely employed in materials like metal pigments in cements, paints, rubber, etc. To avoid the leakage of metals into the marine ecosystems it has been accepted worldwide to decrease the level of antifouling treatments. The most common ways of releasing chromium into the environment are outflowing of water or gas from dumping ground (landfill), chemical plant, trash incinerators, or even occasionally from contaminates rocks or top soils (Greany, 2005).

Vanadium(V): Vanadium is frequently found in crude oils, and is known as a toxic element in the environment. Some of living organisms lodging near the contaminated environments are suffering due to induction of some inhibitory effects in enzymatic activities, breathing disorders, liver and kidney malfunctioning, DNA alteration, and male sterility. Vanadium can be accessed through some environmental species such as plants, vertebrates, algae, and aquatic organisms (Greany, 2005).

Manganese(Mg): Manganese can be reached everywhere in the globe. Higher concentrations of manganese is toxic to animals. Manganese may comprise almost dust particles in the air, small particle in the water as well as solid materials in the soil. Manganese pollutions in the air mainly occur from human industrial activities and fossil fuel combustion (Greany, 2005). Manganese released from human sources can transfer into ground water, surface water and sewage water. Pesticides containing Mg can also enter soils. In plants, Mg has a double-sided symptom, deficiency and toxicity.

Agricultural contaminants

These include application of chemical toxins and manures, utilization of polluted waters and sewages for agricultural purposes and lack of proper management in irrigation. Chemical fertilizers alter soil features, i.e. alleviates soil permeability for water and soil and tighten the soil (Jackson and Alloway,1992). Chemical manures like phosphate fertilizer contain small amounts of cadmium and lead which some of these toxic substances can be absorbed by the plants and eventually enters the human body and causes disease in the long run in case of improper application of these substances. Animal fertilizers that are extensively produced every year, can possibly enter nearby rivers and pollute the river and surrounding soils. In some types of watering patterns, a pond provides for accumulation of water, which leads to: a) leaching of nutrients from the soil and their further carriage into the deep soil where plants cannot access them anymore. Hence, they cause the need for fertilizer again and b) too much evaporation of water causes solutes to deposit on the soil surfaces, making it stiff.

Chemical toxins make the soil polluted too. A number of pests, particularly insects get resistance against pesticide. For this reason, we have to either increase the frequency of spraying or augment the concentration of corresponding toxins. These toxins do not degrade in a simple manner and remain in the soil for many years (Alloway, 1995b; Davies,1995). They might be sublimated without chemical change, absorbed by the surface particles of soil, leave the soil through runoff or leaching or undergo chemical, non-chemical and photodecomposition reactions (Hopenhayn-Rich et al.,1996). In order to rehabilitate and restore the contaminated soil, the following actions might be suggested (Peereboom-Stegeman ,1987; Culbard et al.,1988) : utilization of water to remove pollutants from soils, employment of aerial and chemical solvents, elimination of contaminants by direct burning (Davies,1995), contribution of natural organisms to dissociate the pollutants atoms from soil, addition of material to the soil for preservation and maintenance and prevention of dissemination of pollution to other areas (Alloway, 1995b).

Conclusion

The groundwater contamination either from the natural soil or even from anthropogenic sources is a global concern over public health. Fighting against soil contamination in developing countries is easier than in industrial and developed countries with respect to agriculture, because developing countries are in earlier stages than industrial ones; and chemical manures and phytoxines in developing countries are not consumed as much as in developed ones. Therefore, in these countries, authorities should consider all aspects before taking any effective actions over increasing the level of agricultural and industrial productions of expanding cities. Based on complete understanding of environmental conditions and polluting factors, they should select a way which is proportional to environmental and social conditions of that specific area so that pollution is prevented as much as possible. To do so, the level of farmers' knowledge pertaining to the problems and their aftermath should be enhanced beforehand. To prevent soil pollution, countervailing measures should be in order to improve and reconstruct the contaminated soils, in addition to employing animal manures, as far as possible, rather than chemical fertilizers so that the crops can be consumed without any serious problem. Furthermore, by utilizing chemicals in a more modulated manner, it is possible to not only raise the crop productivity, but also ameliorate the amount of heavy metals in the soil area. As a living and feeding ground for many organisms, and also the determinant basis of ecosystem, soil plays an effective role in all levels of the food chains and food webs in the ecosystem and the environment. Thus, we should preserve this precious asset by proper management and careful surveillance through proper land-use activities.

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Review paper
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CONSERVATION COOPERATION BETWEEN IRAN AND INDIA CAN SERVE AS GLOBAL MODEL

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Abstract

The Asiatic lions (*Panthera leo persica* Meyer, 1826) and the Asiatic cheetahs (*Acinonyx jubatus venaticus* Griffith, 1821) are two critically endangered species of big cats endemic to the Asian continent and currently restricted to distribution in isolated habitats in India and Iran, respectively. Both species had wide distribution in the past, stretching from the Arabian peninsula through Iran and Afghanistan to the vast Indian subcontinent. Unfortunately due to heavy anthropogenic pressure and lack of conservation efforts both species have been wiped out from most parts of their previous range of distribution. Both species have made a come back in these two countries and probably we could now look for a program in slowly repopulating the older habitats of these two species starting with the two current host countries of Iran and India. A Joint Conservation Initiative (JCI) between Iran and India with formal conservation agreements could help procuring the species beyond their current habitats for starting a captive breeding program for Asiatic lions in Iran and Asiatic cheetahs in India. If these programs are successful, it could easily help in releasing the species to selected appropriate habitats in these two countries. A long term success could help in the future repopulation of the Asian habitat that used to be co-inhabited by both these majestic species in the past.

Keywords: *India, Iran, Asiatic cheetah. Asiatic lion, conservation*

Introduction

The recent reports of deaths of over ten critically endangered Asiatic lions (*Panthera leo persica* Meyer, 1826) as a consequence of flash floods in the western Indian state of Gujarat have been an alarming incident involving the loss of precious and vulnerable global wildlife. Although, once in a while incidents of deaths of Asiatic lions are reported; but this is possibly the first time after the conservation of the species was initiated in western India that such a large number of deaths of the majestic Asiatic lions were reported due to a natural disaster. Unconfirmed local reports place the death tolls even higher and tentatively somewhere between 15-19. The sad deaths of the lions are once again a reminder as have been suggested against by several researchers, naturalists, wildlife experts and conservationists in restricting the critically endangered Asiatic lions in one premier natural habitat, called Gir Sanctuary in the western Indian desert state of Gujarat that borders with Pakistan (Chellam and Johnsingh, 1993; Johnsingh et al., 2007; Singh and Gibson, 2011) The local population of Asiatic lions has been estimated to cross the carrying capacity of the Gir Sanctuary and are often reported to be overcrowded and densely populated with shrinking prey bases (Johnsingh et al., 2007; Johnsingh et al., 2007; Singh and Gibson, 2011). The current population if Asiatic lions in India are estimated ~400+ (Johnsingh et al., 2007; Singh and Gibson, 2011). The lion populations are in dire need of relocation to additional habitats within India to reduce the pressure on the Gir Sanctuary.

Challenges of conservation

The stock population of the Gir Sanctuary thus needs to be sub divided into smaller sub populations and distributed under the supervision of the Federal and State Environment and Forest departments, respectively; for successful, long term and sustainable maintenance of healthy lion populations well separated from each group for genetic and ecological purposes. Else the highly vulnerable lion population; although currently showing improvement in terms of numbers, runs the grave risk of undergoing genetic bottlenecks due to higher chances of inbreeding among close family members (Singh et al., 2002; Mukherjee and Borad, 2004). Furthermore, there are also looming dangers of similar natural calamities and catastrophes as mentioned above, issues of unaccounted contagious diseases, reduced prey bases and increased competition for premier hunting grounds and sufficient breeding spaces among different clans inhabiting the Gir Sanctuary; that may also negatively impact the population of Asiatic lion in the near future (Mukherjee et al., 1994; Saberwal et al. 1994). The challenges of anthropogenic pressures in and around the Gir Sanctuary perimeter is also a big issue for the expanding lion population in the region; so that there will be chances of habitat fragmentation as well as human-animal conflicts in the future (Johnsingh et al., 2007; Singh and Gibson, 2011). Unless proactive wildlife management steps are adopted at the earliest to reduce the pressure on the current habitat, the majestic Asiatic lions may be gone before even we realize how vulnerable this critically endangered species is currently (Shankaranarayanan et al., 1997; Singh et al., 2002; Johnsingh et al., 2007).

Iran is an important nation in this regard undergoing similar challenges with Asiatic cheetah (*Acinonyx jubatus venaticus* Griffith, 1821) conservation (Marker and Amy, 2004); the last refuge for the majestic species (Olmsted et al., 1992; Hunter et al., 2007; Charruau et al., 2011) like the Asiatic lion with an isolated habitat in India. The Iranian population of Asiatic cheetahs is falling beyond somewhere around only 100-200 individuals left in the wild (Olmsted et al., 1992; Hunter et al., 2007; Charruau et al., 2011). Most of the Iranian cheetahs are now restricted predominantly in the eastern dry desert region of Iran in different national parks of the provinces like Kerman, Khorasan, Semnan, Tehran, Markazi and Yazd (Karami, 1992; Olmsted et al., 1992; Farhadinia, 2004; Hunter et al., 2007; Farhadinia and Mahmoud-Reza, 2010). Other cheetah sub populations are dispersed sporadically in Iran and are not well recorded (Marker and Amy, 2004). No sightings from adjoining Afghanistan and India have been reported over half a century now (Hunter et al., 2007; Manati and Nogge, 2008). Some unconfirmed reports of Asiatic cheetah observed in the extreme western edges of Baluchistan in Pakistan are available; however, due to extreme poor track record of the country in wildlife and forest conservation, such reports are not given any scientific value (Berwick, 1976; Marker and Amy, 2004; Charruau et al., 2011; Meena et al., 2011).

The path ahead-Joint Conservation Initiative

Historically both species had wide distribution in the past, stretching from the Arabian peninsula through Iran and Afghanistan to the vast Indian subcontinent. Unfortunately, as discussed above due to heavy anthropogenic pressure mostly in the form of unregulated, unrestricted and unmonitored hunting as well as over exploitation for trophy collection; high demands for skins and other body parts; huge drive for personal collections and the zoos around the world and poaching wiped out the species from major parts of their distribution range (Johnsingh et al., 2007; Charruau et al., 2011). Hence, it will be wonderful to expand their habitats beyond their current limited range of distribution and slowly release them to their old habitats where they had once got extinct in the past due to anthropogenic pressure. India and Iran being two biodiverse countries with distinct credentials in successful forest and wildlife conservation; and particularly for their great success in dealing with critically

endangered species need to share their experiences and expertise to help one another to further capacitate their conservation efforts; and empower themselves in the realms of global wildlife conservation. It will be of interest to both nations to initiate a well designed and comprehensive Joint Conservation Initiative (JCI) for captive breeding and release of such critically endangered species to the wild in both India and Iran.

It will be good to bring in some Asiatic lions to Iran for jump starting captive breeding programs in Iran for later releasing them to their native and suitable wild conditions under careful observation and scientific monitoring. Similarly, Asiatic cheetahs could be introduced into India from Iran thereby attempting to introduce the lost species back to the Indian subcontinent. Although the African lions and cheetahs are much plentiful in number (Ott-Josh et al., 1987; Saleh et al., 2001; Charruau et al., 2011); but since they are widely geographically separated from their Asiatic cousins by several millions years back in the history of evolution; the best available option is to experiment carefully and with sincerity with the Asiatic species only in the Asian continent.

Conclusion

If such joint initiatives (or JCI) are made successful by both Iran and India these could serve as global model of international cooperation in wildlife conservation; and both these proud nations could become pioneers in such initiative (JCI) for the whole planet. If these programs are successful, it could easily help in releasing the species to selected appropriate habitats in these two countries. A long term success could help in the future repopulation of the Asian habitat that used to be co-inhabited by both these majestic species (Asiatic lions and Asiatic cheetahs) in the past. Anthropogenic pressures in the past has removed both these species from their original range across Asia; and hence it will be the responsibility of humans again to join forces and efforts together to support an initiative of slowly returning these top predatory species back into their wild Asiatic habitats. Joint Conservation Initiative could serve as a successful bridging platform in the process of reintroduction of Asiatic lions and cheetahs to their broader habitats.

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Original scientific paper
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SPATIAL VARIABILITY OF SOIL-PLANT PARAMETERS UNDER DIFFERENT SALINE WATER TREATMENTS

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Abstract

Spatial pattern and interdependence of different soil and plant parameters were examined in green bean field experiment carried out at the Mediterranean Agronomic Institute of Bari (MAIB), Italy. The study aimed to identify the spatial distribution of soil and plant parameters and their relationship at transects scale. The experiment consisted of three transects of 30m length and 4.2m width, irrigated with three different salinity levels (1dSm^{-1}, 3dSm^{-1}, 6dSm^{-1}). Soil measurements (electrical conductivity and soil water content) were monitored along each transect in 24 sites,1 meter apart by using TDR probe installed vertically at soil surface. Water storage was measured by using Diviner sensor for calculating directly the evapotranspiration fluxes along the whole soil profile under the different salinity levels imposed during the experiment. In the same 24 sites, crop monitoring involved measurements of Leaf Area Index (LAI), Osmotic Potential (OP), Root length Density (RlD) and Evapotranspiration fluxes (ET). Soil and plant properties were analyzed using both classical and geostatistical methods which included descriptive statistics, semivariograms and cross-semivariograms. Results indicated that moderate to large spatial variability existed across the field for soil and plant parameters, especially under the 6dSm^{-1} salinity treatment. A relatively satisfactory fit of the experimental cross-semivariogram was obtained for the 6dS^{-1}, thus indicating similar spatial structures of the pairs of compared variables. By contrast, the experimental cross-semivariograms observed under the 3dS^{-1} treatment indicated no significant correlation structure between the compared variables. Overall, the results observed in the 3dSm^{-1} were not significantly different from those obtained in the 1dSm^{-1} transect and suggested a general insensitivity of the crop response to those levels of salinity.

Keywords: *Soil and crop properties, Spatial variability, Semivariogram, Cross-Semivariogram*

Introduction

Salinity is a major problem in agriculture sector in arid and semiarid regions. The accumulation of salts over time in the root zone limits the water uptake of plants by reducing the osmotic potential and thus the total soil water potential (Shannon *et al.*, 1994; Sheldon *et al.*, 2004;Corwin and Lesch, 2005). Salinity is rarely uniform with depth and usually varies with time at field scale. The soil physical and chemical factors such as soil texture, water content and salinity distribution in the soil profile are the most dominant influences on yield and growth variability (Dixit and Deli, 2010).

Salinity has been extensively reported to have negative impacts on the majority of arable crops and classified them as sensitive, moderately sensitive and high sensitive crops (Sheldon *et al.*, 2004). Many laboratory and field experiments have been conducted to evaluate crop salt tolerance under uniform salinity along the soil profile. Comprehensive reviews of such studies are well documented by (Maas and Hoffman, 1977; Maas, 1993). However, salinity is rarely uniform in space on both the vertical and the horizontal direction. The spatial variability of soil salinity with water content have shown dominant effects on crop yield

variability (Dixit and Deli, 2010; Coppola *et al.*, 2014). However, the effects of soil salinity on the physiological crop parameters and their spatial variation across field still require depth analysis. Spatial variation of various soil and plant properties may be was studied by using classical statistics and geostatistics intensively. Geostatistics may provide useful information for assessing spatial variability of soil properties and has increasingly been used by soil scientists and researchers in recent years (Jabro *et al.*, 2006; Jabro *et al.*, 2010). Semivariograms and cross-semivariograms have been used to characterize and model spatial variance of data to assess how data points are related with separation distances. There are many studies in the literature on the use of geostatistic analysis to describe the spatial variability of single soil properties (López-Granados *et al.*, 2002; Jabro *et al.*, 2006; Jabro *et al.*, 2010; Yang *et al.*, 2011; Fathi *et al.*, 2014a). However, rarely these studies have reported information on the spatial variability of soil parameters (i.e., water content and electrical conductivity) and the corresponding variability on plant growth at field scale under different water salinity levels.

Therefore, the objectives of this study were to apply geostatistics approach to characterize field scale spatial variability of selected soil-plant properties and to explore the spatial interdependence between soil-plant parameters using cross-semivariogram analysis.

Materials and methods

The experiment was carried out at the research station of MAIB in 2013 south-east of Italy. The soil was pedologically classified as ColluvicRegosol, consisting of a silty loam layer of an average depth of 70 cm on a fractured calcarenite rock. The experiment consisted of three transects of 30 m length and 4.2 m width, equipped with a drip irrigation system, consisting of fourteen dripper lines at 35 cm distance and a distance among drippers along each line of 20 cm, with dripper discharge of 2 l/h. The transects were irrigated with three different salinity levels ($1dSm^{-1}$, $3dSm^{-1}$, $6dSm^{-1}$) by using calcium chloride ($CaCl_2$). Each transect consisted of seven rows of green beans crop, 70 cm apart and plants in each row 40 cm apart. The irrigation volumes were calculated according to the water storage measured by a Diviner 2000 sensor. Twenty-four access tubes, 1 m apart, were installed along the middle line of each transect at 60 cm depth to monitor soil water content using Diviner 2000 sensor. Diviner readings were taken at 10 cm depth intervals up to 60 cm just before and two hours after every irrigation applications. The difference between water storage at field capacity and the storage measured just before irrigation allowed calculating the irrigation volumes to bring the soil water content in the root zone to the field capacity. The apparent electrical conductivity was monitored during the growing season by inserting a TDR probe vertically at the soil surface in 24 sites along the middle line of each transect. Furthermore, plant measurements (e.g., Leaf area index, Transpiration rate, Root length density, Osmotic potential and yield) were carried out to evaluate the physiological state of plants, on the same 24 sites already identified for soil measurements. As crop responses to salinity depend on the electrical conductivity of soil solution (EC_w), a calibration procedure of TDR was also requested for translating ECb values measured by TDR to the corresponding ECw values. In this study, TDR probe calibration was carried out in the laboratory according to the procedure described by Mallants *et al.* (1996).Geostatistical analysis, including semivariogram, cross-semivariogram model fitting procedures, were carried out using GS^+ (Gamma Design Software, 2004, Geostatistics for the Environmental Sciences, St. Plainwell, Mich.) to evaluate the degree of spatial variability of soil and plant parameters.

Results and discussion

Table 1 shows the summary of the statistics of the measured soil and plant parameters. The coefficient of variation (CV) values of measured soil and plant properties in the three different

treatments ranged between 29.29% for electrical conductivity of soil solution (ECw) and 7.17% for soil water content (θ) in the transect treated at $1dS^{-1}$. The standard deviation (SD) of all parameters exhibited the highest values in transect treated with $6dS^{-1}$ saline water, except in the case of LAI. Moreover, the soil and plant data were all normally distributed (with a skewness between 1 and -2) and therefore no transformation was used for geostatistical analysis.

Table1. Summery statistic for selected soil and plant parameters

Soil and plant parameters	Mean	Min	Max	CV%	SD	Skewness	Kurtosis
ECw-$1dS^{-1}$	2.35	0.87	3.69	29.29	0.69	-0.41	-0.33
ECw-$3dS^{-1}$	5.25	3.77	7.07	13.98	0.73	0.16	0.85
ECw-$6dS^{-1}$	6.26	1.64	8.26	23.67	1.48	-1.36	2.12
θ-$1dS^{-1}$	0.25	0.22	0.29	7.17	0.02	0.36	-0.73
θ -$3dS^{-1}$	0.22	0.20	0.27	7.86	0.02	0.75	0
θ -$6dS^{-1}$	0.22	0.20	0.27	11.06	0.03	0.44	-1.21
ET-$1dS^{-1}$	0.51	0.36	0.73	20.58	0.10	0.61	-0.53
ET-$3dS^{-1}$	0.53	0.33	0.76	22.51	0.12	0.41	-0.80
ET-$6dS^{-1}$	0.48	0.27	0.75	26.99	0.13	0.25	-1.02
LAI-$1dS^{-1}$	0.63	0.35	0.91	25.19	0.16	0.17	-0.78
LAI-$3dS^{-1}$	0.66	0.35	0.99	22.14	0.15	0.47	0.74
LAI-$6dS^{-1}$	0.52	0.29	0.68	18.23	0.10	-0.23	-0.79
OP-$1dS^{-1}$	0.91	0.78	1.12	9.18	0.08	0.81	-0.18
OP-$3dS^{-1}$	1.04	0.74	1.51	15.18	0.16	0.84	-0.22
OP-$6dS^{-1}$	1.15	0.82	1.39	13.73	0.16	-0.34	-0.83
RlD-$1dS^{-1}$	0.52	0.29	0.68	18.23	0.10	-0.23	-0.18
RlD-$3dS^{-1}$	2.14	1.28	2.65	18.17	0.39	-0.75	-0.22
RlD-$6dS^{-1}$	1.84	1.13	2.56	21.06	0.39	0.01	-0.83

The semivariograms calculated for soil and plant data (EC_W, θ, ET, LAI, OP, RlD) are depicted in Figure 1.

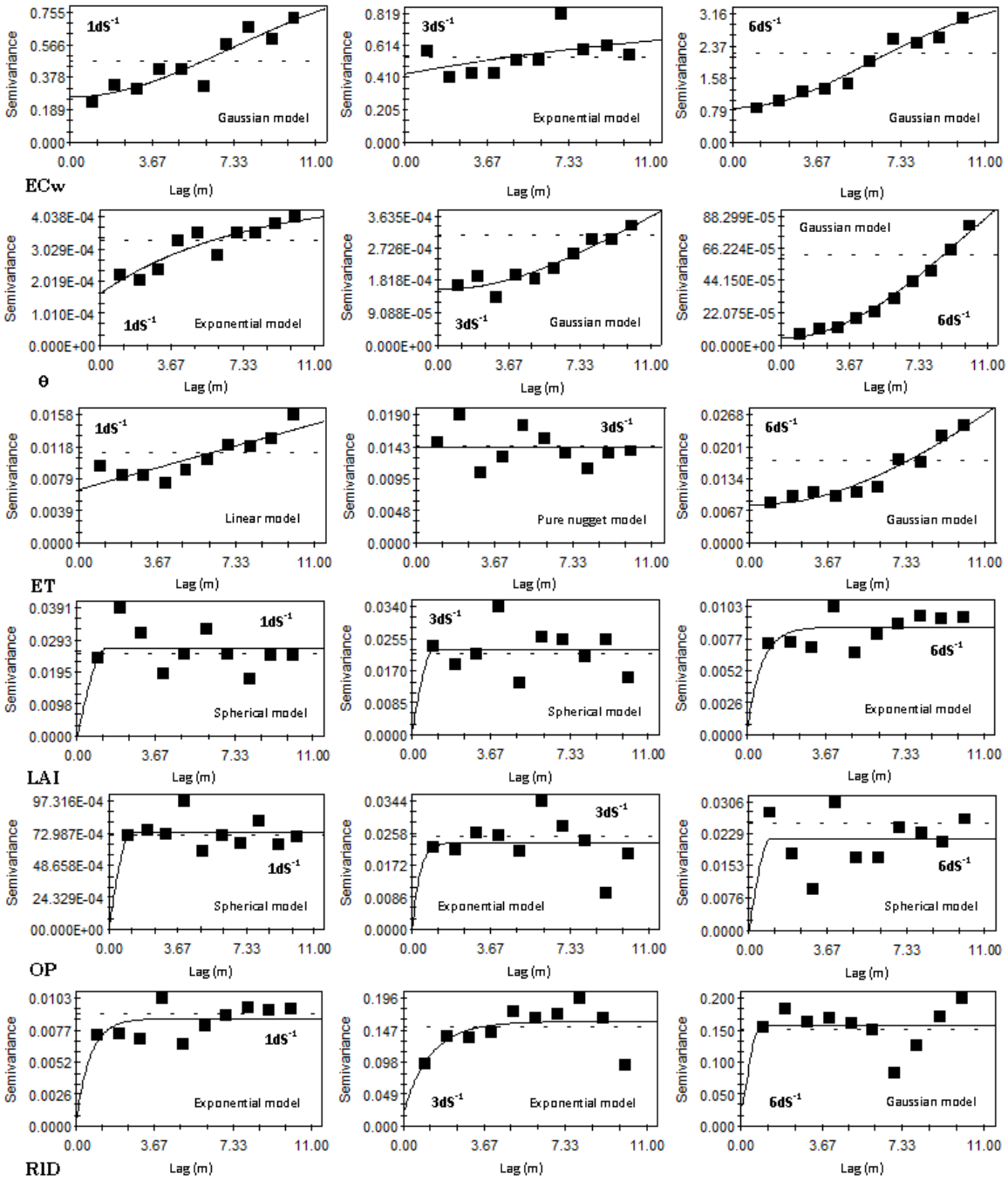


Figure1. Semivariogram models of soil and plant parameters.

Semivariogram coefficients for each soil plant parameters with the best fitted model are listed in table 2. The R^2 values in table 2 show that models fit the experimental field semivariogram data very well for soil parameters (EC_W,θ), as well as for evapotranspiration (ET) in transects treated with $1dS^{-1}$ and $6dS^{-1}$. Simultaneously, the Residual Sum of Squares (RSS) values were very small for semivariogram models of soil and plant parameters monitored in this study, except for ECw. The ratio between nugget (C0) and sill (C0+C) (the so called nugget ratio) was calculated for soil and plant properties and used to investigate the degree of spatial dependence across the experimental field. Many authors reported that if the nugget ratio is ≤

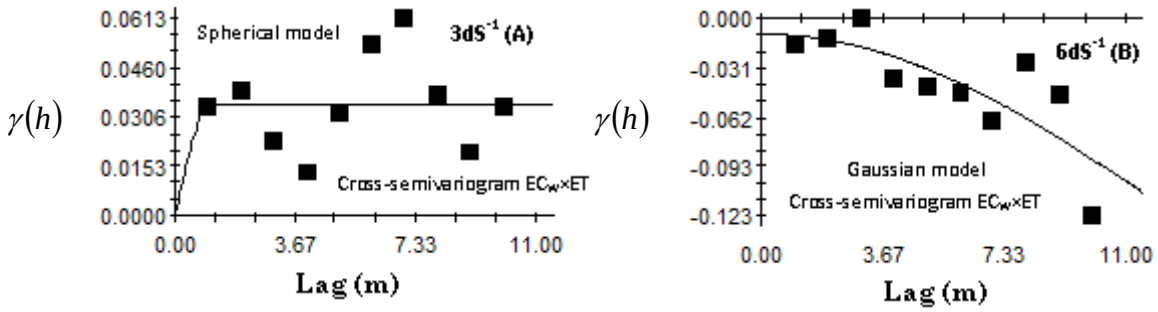
0.25, the variable should be considered to be strongly spatially dependent; if the nugget ratio lies in the range between 26 and 75% the variable has to be considered moderately spatially dependent; finally, if the nugget ratio is greater than 75%, the variable has to be considered weakly spatially dependent (Cambardella *et al.*, 1994; Mallants *et al.*, 1996; López-Granados *et al.*, 2002; Jabro *et al.*, 2010; Fathi *et al.*, 2014b). From the values of nugget ratio for the semivariograms in Figure1, it seems that that there is moderate spatial dependence of EC_w and ET at transects treated $1dS^{-1}$ and $3dS^{-1}$Also, a moderate spatial dependence was found for θ in the transect treated with $1dS^{-1}$. On the contrary, the nugget ratio for the other soil and plant parameters indicated weakly spatial dependence in all the three transects (see table 2).

Table2. Coefficients of the theoretical semivariogram model of soil and plant parameters

Soil and plant parameters	Model	Nugget,C_0	Sill,C_0+C	Nugget Ratio C_0/(C_0+C)	Range A(m)	RSS[a]	R^2
ECw-$1dS^{-1}$	Gaussian	0.264	0.968	0.727	10.06	0.0400	0.84
ECw-$3dS^{-1}$	Exponential	0.433	0.868	0.501	17.04	0.0942	0.23
ECw-$6dS^{-1}$	Gaussian	0.839	3.657	0.771	8.36	0.191	0.96
θ-$1dS^{-1}$	Exponential	0.00017	0.00046	0.642	7.07	$7.289*10^{-9}$	0.82
θ -$3dS^{-1}$	Gaussian	0.00016	0.00070	0.777	15.87	$3.734*10^{-9}$	0.90
θ -$6dS^{-1}$	Gaussian	0.00005	0.00210	0.976	15.25	$7.458*10^{-9}$	0.98
ET-$1dS^{-1}$	Linear	0.00662	0.01387	0.523	10	$1.535*10^{-5}$	0.73
ET-$3dS^{-1}$	Pure nugget	0.01423	0.01423	1	10	$6.247*10^{-5}$	0
ET-$6dS^{-1}$	Gaussian	0.00790	0.07870	0.9	19.71	$1.767*10^{-5}$	0.93
LAI-$1dS^{-1}$	Spherical	0.00003	0.02676	0.999	1.36	$3.552*10^{-4}$	0.01
LAI-$3dS^{-1}$	Spherical	0.00176	0.02252	0.922	1	$3.052*10^{-4}$	0
LAI-$6dS^{-1}$	Exponential	0.00074	0.00868	0.915	0.68	$1.211*10^{-5}$	0.14
OP-$1dS^{-1}$	Spherical	0.00045	0.00734	0.939	1.13	$9.898*10^{-6}$	0
OP-$3dS^{-1}$	Exponential	0.00092	0.02344	0.939	0.36	$3.437*10^{-4}$	0
OP-$6dS^{-1}$	Spherical	0.00001	0.02172	1	1	$3.444*10^{-4}$	0
RlD-$1dS^{-1}$	Exponential	0.00074	0.00868	1	0.68	$1.211*10^{-5}$	0.14
RlD-$3dS^{-1}$	Exponential	0.02050	0.16000	0.872	1.24	$6.446*10^{-3}$	0.36
RlD-$6dS^{-1}$	Gaussian	0.03150	0.15700	0.799	0.47	$9.066*10^{-3}$	0

[a] RSS is the residual sums of squares for the theoretical semivariogram models.

The graphs in Figure 2 (A, B, C and D) depict the cross-semivariograms calculated for selected pairs of soil and plant parameters (ECw vs. RLD and ET) in the $3dS^{-1}$ and$6dS^{-1}$ salinity treatments. Cross-semivariograms were calculated to identify any possible effects of soil salinity and its affect on the root length density and evapotranspiration fluxes, as well as their spatial interdependence. In this study different theoretical cross-semivariogram models have been used (namely Gaussian, Exponential and Spherical). Table 3 depicts the parameters obtained for each of the model used.



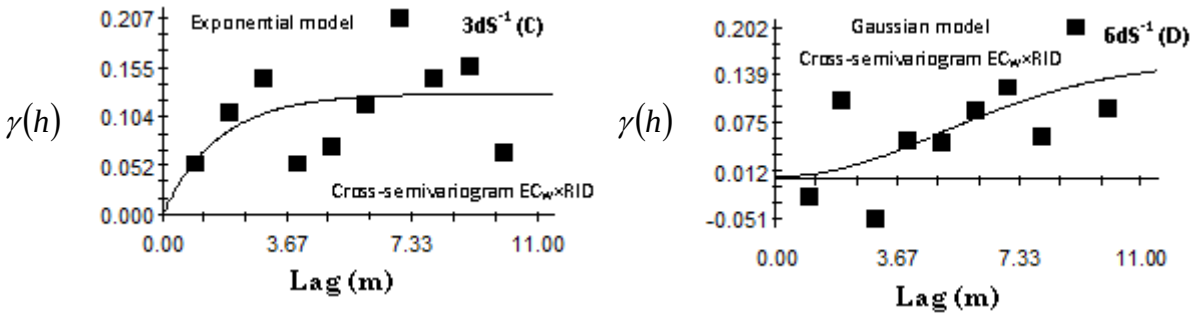


Figure2. Cross-semivariogram models of selected soil and plant parameters.

According to the R^2 values, a relatively good fit of the experimental cross-semivariogram was obtained for all the cases at $6dS^{-1}$, thus indicating similar spatial structures of the pairs of compared variables. By contrast, the corresponding values for the $3dS^{-1}$ treatment indicate an unsatisfactory fit and thus no significant correlation structure between the compared variables. Overall, the results observed in the $3dSm^{-1}$ were not significantly different from those obtained in the $1dSm^{-1}$ transect (data not shown), and suggested a general insensitivity of the crop response to those levels of salinity. As for the $6dSm^{-1}$ treatment, according to the criteria suggested by Cambardella *et al.* (1994) to evaluate cross-correlation between two variables, the cross-semivariograms of the figure 2 suggest positive spatial interdependence at all lags except the lag=1 and 3 (ECw×RlD). On the contrary, a negative spatial interdependence was observed between electrical conductivity of soil solution ECw and ET (see figure 2B). These conflict results of spatial interdependence between some soil and plant parameters might be related with the dynamic of salt and water in the soil profile.

Table3. Coefficients of the theoretical Cross-semivariogram model of soil and plant parameters

Soil and plant parameters	Model	Nugget, C_0	Sill, C_0+C	Nugget Ratio $C_0/(C_0+C)$	Range A(m)	RSS[a]	R^2
ECw×ET $3dS^{-1}$	Spherical	0.00010	0.03490	0.00286	1.12	$1.905*10^{-3}$	0.10
ECw×ET $6dS^{-1}$	Gaussian	-0.01000	-0.32700	0.03058	18.79	$4.104*10^{-3}$	0.61
ECw×RlD $3dS^{-1}$	Exponential	0.00010	0.12820	0.00078	1.66	0.0191	0.17
ECw×RlD $6dS^{-1}$	Gaussian	0.00270	0.15540	0.01737	7.13	0.0269	0.42

[a] RSS is the residual sums of squares for the theoretical semivariogram models.

Conclusion

The spatial variability and the interdependence between different soil and plant parameters were explored at transect scale under three different salinity applications by using classical statistics and geostatistical approaches. The results from both the approaches indicated that moderate to large spatial variability existed in this study across the field for soil and plant parameters especially under the $6dSm^{-1}$ salinity treatment. In general, the statistical approach used proved helpful to establish the relationship between the spatial distribution of soil water salinity and the corresponding variability of effects on canopy development and yield indices of bean based on different soil and crop sensors. Field scale variability of soil salinity and crop response have been generally approached by classical randomized blocks schemes allowing for calculating the main statistics of that response as related to the average and variance of soil salinity. By contrast, our study used close monitoring sites along continuous

extended transects rather than randomized blocks. This way, the spatial variability of the relationship soil salinity-crop response were analyzed continuously in the space and the spatial correlation (which cannot be observed in randomized blocks) can be used as an additional and important information for understanding the mechanisms of plant response to salinity.

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A MODERN MANAGEMENT MODEL FOR IRRIGATION SYSTEMS

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Abstract

Collective irrigation systems are generally characterized by large areas to be served and large volumes of water to be distributed - water, which may be in short supply in the utilization zone. Improving the efficiency of these systems is possible through the use of automation and remote control, which is necessary particularly where there is a scarcity of water. Automation allows the water manager to have knowledge of the plant operational data in near-real time, facilitates a timely response to demand and ensures supply within the range of predetermined irrigation parameters. For large irrigation networks in open field, the implementation of these delivery systems to end users may require major capital in terms of both set-up and maintenance costs. These funds are not always available to managing institutions and must be justified by careful cost-benefit analysis. A solution to this problem has been implemented in some irrigated plains of the Volturno valley, under the jurisdiction of Consorzio Sannio Alifano. By adopting an appropriate management model, irrigation plant operation using remote control and automation has been achieved with low overall investment. To avoid over-consumption of water and rationalize the management of irrigation networks, an automated decision support system was developed, using state-of-the-art, user-friendly software, implementing GIS models and remote sensing data through satellite imagery, with users having only to book irrigation time. The results of this approach have been positive and will be discussed along with the overall system description.

Keywords*: Management irrigation systems, irrigation advisory services, water saving, remote control, remote sensing.*

Introduction

There are several stakeholders who can take action to improve the efficiency of the irrigation sector. First of all managing institutions of collective irrigation systems, i.e., drainage and irrigation authorities (Consortia), who are tasked with providing accurate estimate of irrigation requirements as well as modern and efficient irrigation system. They are also responsible for the management of the water distribution adapted to farmers' needs. Secondly, there are irrigation users/farmers, who access irrigation systems and utilize them with methods suitable to different environmental conditions of their crops as well as a variety of social conditions. With respect to management institutions, it may be argued that the collective irrigation in a region made by Consortia is typically organized in one of the following ways:

“delivery in turn and time” (ITT) - the delivery of the water to each user is performed on a rotational schedule at assigned time intervals (turns), with fixed flow rate to the farm module and for an established duration (time);

“delivery on-demand”(DOD) - users have the freedom to water the fields in times of their choosing only constrained by limiting a water flow rate to an assigned module (farm module);

“delivery on-booking”(DOB) - water delivery is made to users who are authorized according to requests received (bookings), within an assigned farm module. The date and time are set according to constraints of the irrigation plant which vary during the irrigation season.

Although differing terminology is sometimes used to characterize the above water distribution modes (Reploge and Merriam, 1980), most irrigation Consortia fall into one of these categories. Irrigation collectives systems are typically characterized by considerable surface areas over which to distribute irrigation service (typically on the order of thousands of hectares), and large volumes of water to be distributed, which is not always available in the area of use.

These collective distribution systems are both complex and difficult to control down to the individual delivery points and, thus, can have both high technical complexity and high capital and operating costs. In recent years, due to the reduction of available water resources, investment in the irrigation field have been directed mainly away from networks of open channels for water delivery and towards networks of pressurized pipes, i.e., system modernization rather than irrigation expansion. An irrigation network, while a necessary prerequisite for using more efficient irrigation methods, i.e., sprinkler heads or localized influx such as micro- and drip-irrigation, cannot alone ensure optimal use of water resources. This is particularly true if optimization is understood in terms as obtaining maximum crop production with the lowest consumption of irrigation water compatible with ecosystem constraints (Lamaddalena et al., 2000).

Other factors obviously intervene in the optimization process. For managing bodies emphasis is currently placed on the irrigation infrastructure, using automation and remote control, and on the management of irrigation plants, using of data from automation systems, remote monitoring, etc. The implementation of a system of automation and remote control of an irrigation network puts in the hands of the manager of the system a large amount of data. Unfortunately, until very recently, such data is often of marginal utility to the system operator. In many cases, in order to overcome complexity in their management, irrigation networks have been designed for a DOD approach, allowing users the freedom to irrigate, within a given farm module, at times not otherwise committed. This distribution system, as opposed to the more restrictive ITT system, would appear to represent the best possible solution to water distribution within an irrigation system at least for the end users, who buy the freedom to organize the irrigation practices in the time and manner most suitable to them (Merriam, 1987). The DOD schedule does not limit the frequency, rate and duration of irrigation water applications. This degree of flexibility requires large capabilities of the irrigation system in terms of water storage and pipeline diameter to meet theoretical peak demand, has two main drawbacks from the perspective of the system as a whole. For the managing body, this approach makes it impossible to know both who is irrigating at any given time and where irrigation is being performed, unless there is irrigation system remote control at the last delivery point. In spite of a perceived advantage, the end user in such a system has the risk of not being able to draw from the water network when required either because of a lack of water or reduced water pressure. This risk is, in fact, inherent in the design of any irrigation design based on solely probabilistic methods. The DOB system avoids these problems by providing an “on-demand” schedule controlled in advance by the water manager. It gives the final users assurance of good and safe irrigation and prevents excessive waste of water.

At this point, a clear picture of the needs of both parties in any efficient irrigation system should be evident. The managing body needs to have the knowledge in as close to real time as possible of data with respect to the irrigation plant in order to ensure continuity of supplied to users within predetermined parameters. Such data include flow rate and pressure. By the same token, the individual user must be able to draw the required irrigation water from the delivery outlets in terms of total amount, flow rate and pressure to be able to meet the water requirements of his crop(s) at any given point in time. The following example illustrates how these two needs have been met by the Consorzio Sannio Alifano.

The area of study is shown in Figure 1, encompassing a region to the right of the Volturno River and, specifically, the plains of Vairano Patenora, Riardo, Pietramelara and Pietravairano, in the Province of Caserta. The aim of this paper is highlight those aspects of the study that can be generalized, in order to provide stimulus for both discussion of developing system needs and refinements as well as to illustrate the potential application of this approach to other management bodies.

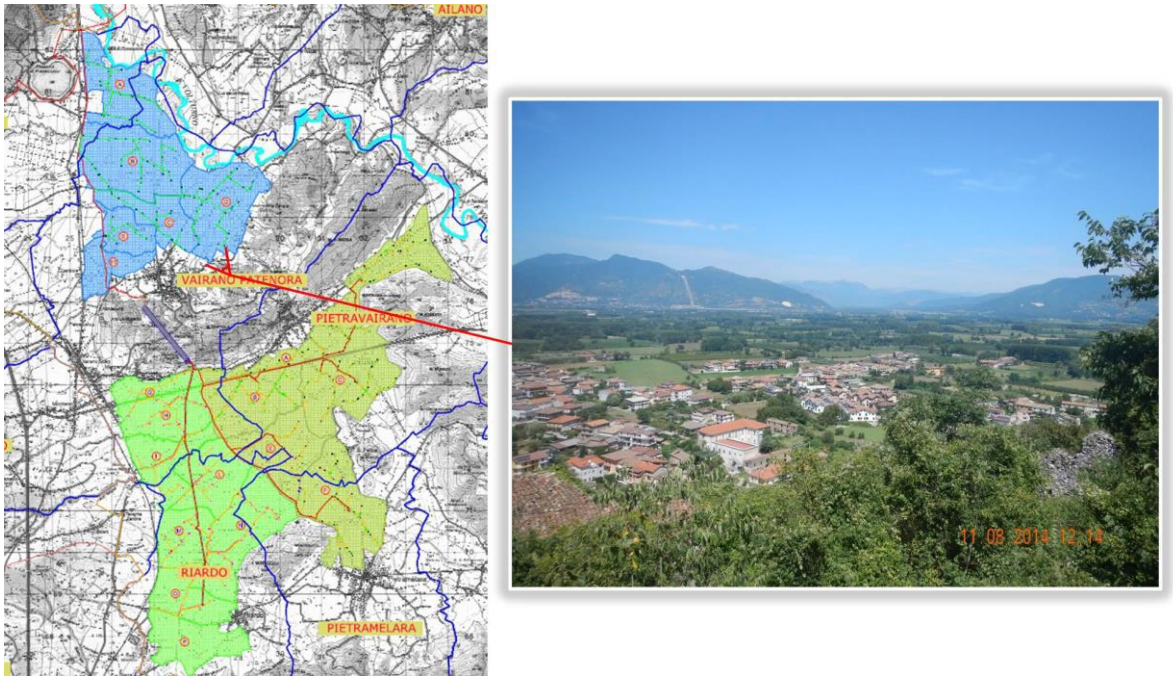

Figure 1. District plains of Vairano Patenora, Riardo, Pietramelara, Pietravairano. The area served by the Consorzio to the right of the Volturno River is shaded in this map.

Materials and methods

The irrigation system encompassing the area illustrated in Fig. 1 has the overall schematic organization illustrated in Figure 2.

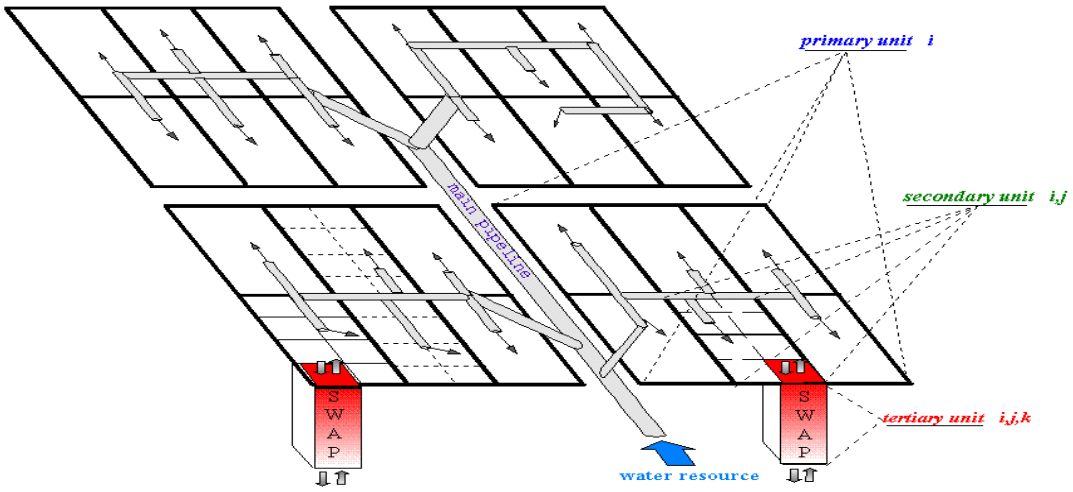

Figure. 2. Schematic organization of irrigation network implemented by the Consorzio Sannio Alifano.

Water flows from the main distribution system to primary units or water districts which are in turn subdivided into any number of secondary units with an average size of 10 hectares, all controlled by the Consorzio. The system is then further subdivided into any number of tertiary units. These could be individual fields under the control of a number of individual users or subdivisions within a single farm encompassing different crop types and/or distribution modules.

The distribution system itself is a network of pressurized piping and the total surface served by this system is 5,690 hectares. Water availability of the entire system, calculated as continuous 24 hours at a unitary flow rate, covering the period at the irrigation tip, is 0.30 l/s/hectare, with a daily irrigation schedule of 16 out of 24 hours. The flow rate at each farm module is limited up to 10 l/s.

The main hydraulic nodes of the network and all the delivery groups installed at the start of each secondary unit are restricted through a remote-controlled system (see Figure 3). The delivery groups are equipped with an electronic card for activation and metering system, called AQUACARD or HYDROPASS (commercial brands), which controls hydrovalve opening and logs irrigation duration, date and volume. The automated delivery group can also be programmed to limit the withdrawals to a fixed amount (i.e. related to the payment of fees or due to overall shortage of water resources).

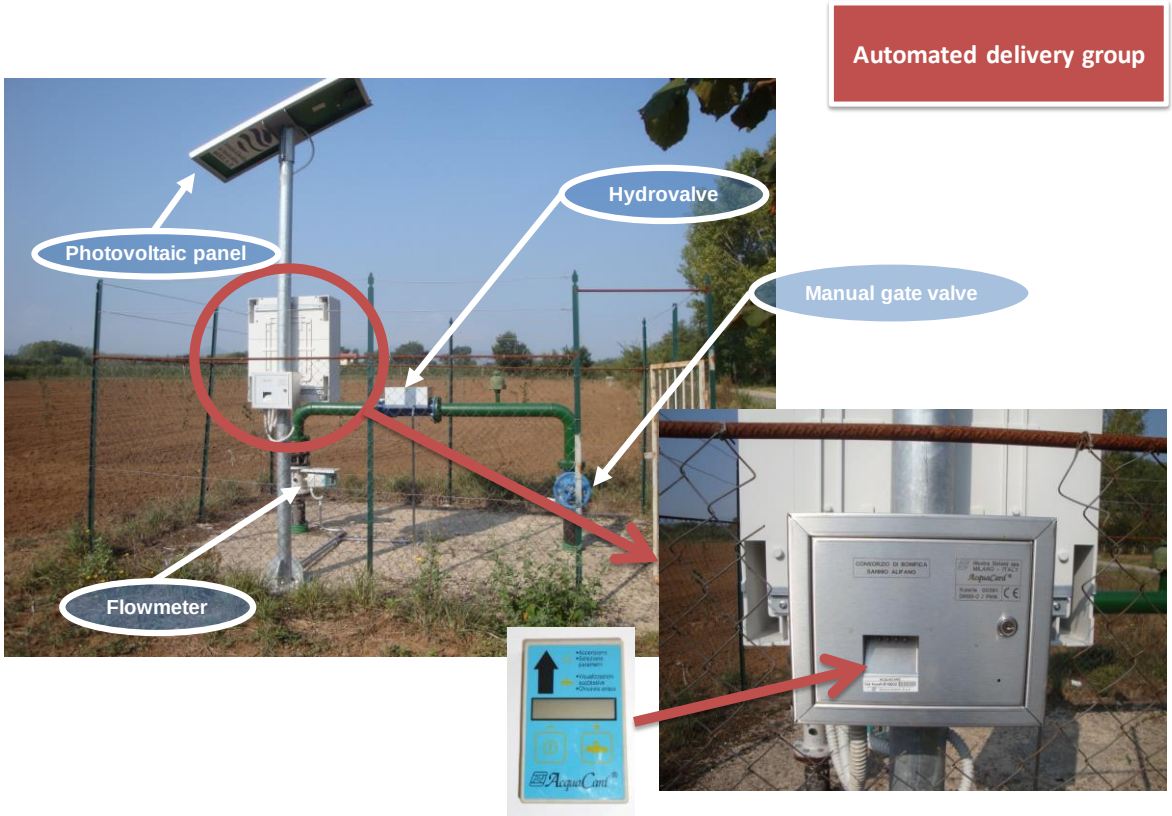

Figure. 3. Remote control of an automated delivery group implemented by the Consorzio Sannio Alifano. Water distribution to users is regulated by means of an “electronic card”.

In the area of study, each secondary unit was equipped with a network of PVC pipes of 125 mm in diameter single and arranged for operation of an outlet (hydrant) to time, enabling the Consorzio to guarantee to each user in every secondary unit a satisfactory degree of freedom in irrigation time and amount. This design choice allowed the Consorzio, through the remote control of the secondary unit, to have real-time knowledge of the user who has opened the group as well as all the data relating to withdrawals from the network. This is due to remote control of the automation equipment by electronic card that is at the start of the secondary unit

(see Figure. 2 and 3). Even in water districts organized differently with the possibility of withdrawals from a large number of hydrants at the same time (i.e., systems divided into larger secondary units), it is possible for the managing body have an adequate knowledge of the functioning of the irrigation system in a manner that ensures satisfactory performance to the end users of the system, without an exorbitant investment in remote control of every automated delivery group implemented at its ultimate limits (i.e. the hydrant at the service of every single parcel, the tertiary units of Fig. 2). This is accomplished by focusing on an appropriate model of irrigation plant management. In this case, the system must be equipped with a remote control system at least until at the start of each secondary unit and with automated delivery group (no remote-controlled) on each hydrant at the service of farm parcels.

Results and Discussion

The management model adopted by the Consorzio Sannio Alifano for the afore mentioned irrigation system is based upon a decision to organize the management of the distribution on the basis of the "reservations" of users. In order to ensure optimum operation of a pressurized distribution network, the managing body must have knowledge of the operation of the irrigation system, a knowledge of user requirements, and knowledge of the flow, pressure and volume of water used at the various distribution points within the system.

To do this, the Consorzio adopted a reservation-based water distribution system with a program for the management of user irrigation reservations. In the case study, the system was equipped with a remote-controlled system at the start of each secondary unit, including the remote control of each automated delivery group. The management approach was based on compiling and updating information on water rights. This simply means that the managing body had an updated record of water rights and land-use cover map. This information, at cadastrian level, was given to Consorzio by the farmers and/or landowners of the parcels to be irrigated. Once having the reference point of the parcel, it was then identified on cadastral maps and, by means a Geographic Information System (GIS), linked to the irrigation network and to the corresponding delivery group. The Consorzio Sannio Alifano began this work based on a 2013 GIS inventory of irrigated plots, which is regularly updated every year. Using this system, the water delivery to each parcel was managed in an easy and rational way.

The most important aspect of the overall water management system was the incorporation of a Geographic Information System (GIS). The GIS made it possible to continuously display real time operation of the irrigation system on the basis of "reservations" accepted and the data collected by the remote control system (see Figure 4). Because they are related to the “reservations” of the users, the GIS data are only an approximation of reality, but integrating them with the data from the remote control system, it is possible to obtain an overall irrigation system picture that is very nearly so. These system overviews are especially useful to the Consorzio, since they are obtained using a remote control system less complex, sophisticated and expensive than otherwise obtainable, implementing automated delivery groups on every individual delivery point, i.e., every hydrant at service of the parcels.

While the implementation of an AQUACARD or HYDROPASS system, connected via mobile phone modem to the remote control centre, is expensive and requires additional manpower, particularly for maintenance, the data acquired from the system adopted by Consorzio was used to verify the degree of system reliability, since managers were able to check the differences between the data derived from "reservations" with actual consumption by end users through automated delivery groups at any time of the irrigation season. This was done in many ways: directly by remote control system or, in case of its temporary outage, by targeted interventions of Consorzio field workers upon water delivery groups, or by reading user irrigation cards in the offices of the Consorzio when irrigation reservations are made.

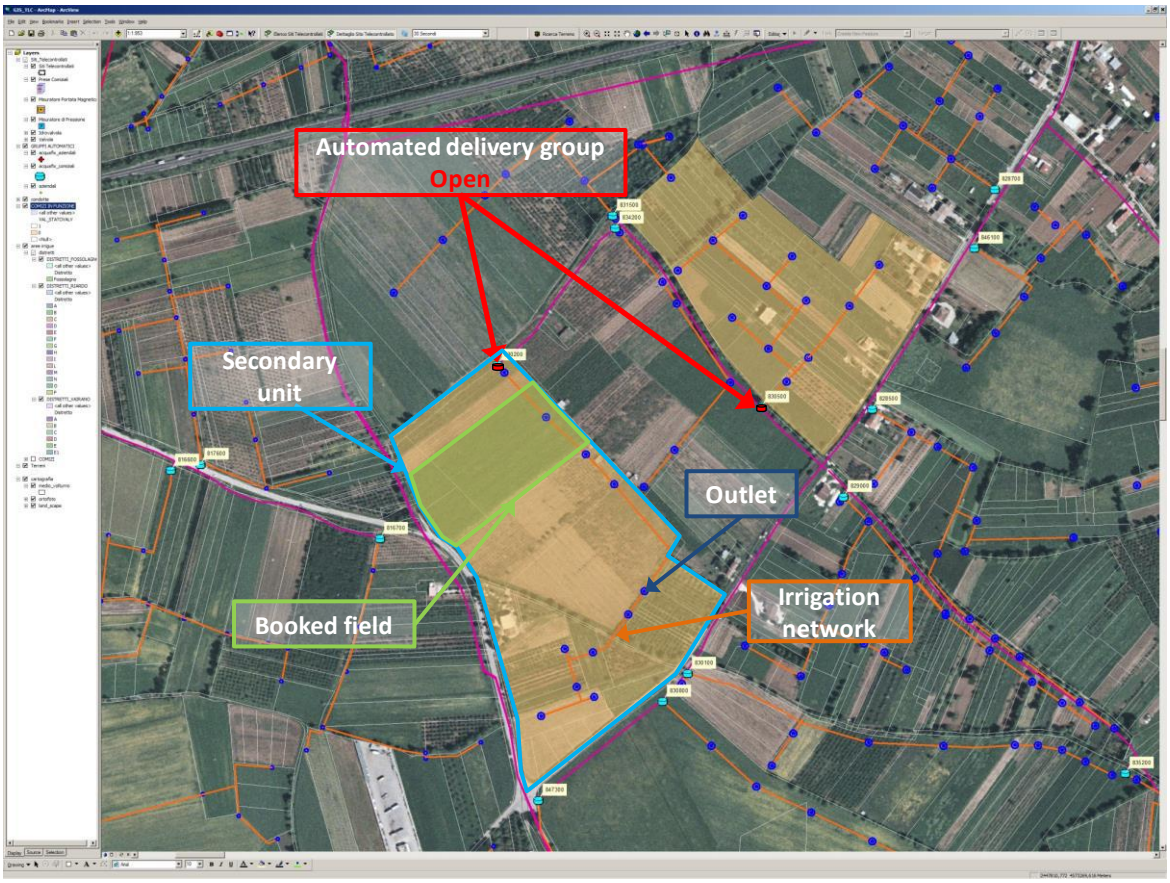

Figure. 4. An overview of the system of water delivery by Consorzio Sannio Alifano.

If the water management body has a "water balance model," it can integrate model predictions into its overall management of the irrigation service. In the case of Consorzio Sannio Alifano, the model used is called IRRISAT, based on the use of satellite imagery (D'Urso et al., 2010; Vuolo et al., 2015). Using such a model, it was possible for the management body to recommend timely irrigation to users or revise water volumes that were more appropriate for the particular crops being grown. This "irrigation advice" permitted maximization of irrigation effectiveness for the user at individual parcel level and optimized water usage within the irrigation district as a whole.

IRRISAT (www.irrisat.it), in particular, was an innovative irrigation support system, such as an "irrigation advisory", based on the use of satellite imagery to monitor the development of crops in the field; it estimates the max amount of irrigation water to be applied in a given time interval and it allows mapping of irrigated areas, such as detecting unauthorized irrigation is also possible. The system integrated satellite sensor data (in the visible and near infrared wavelengths) and *in-situ* agro-meteorological measurements to produce maps of crop water requirements (see Figure. 5).

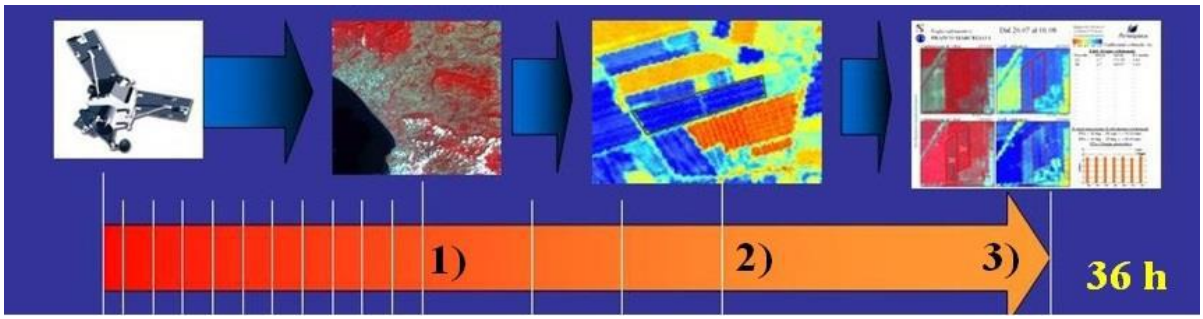

Figure. 5. Schematic of data integration using IRRISAT. Data are available to the users within 36 hours from the satellite pass.

The IRRISAT approach has proven that economic benefits generated by such advisory services are able to fully repay the initial investments, creating advantages for environment and opportunities for all the users of water resources (Vuolo et al., 2015). Accordingly, IRRISAT has been deemed a “best practice” for agricultural applications by EURISY (http://www.eurisy.org/good-practice-campania-encouraging-the-sustainable-use-of-irrigation-water-in-the-region_85) and by the International Selection Committee of the call for “Best Sustainable Practices on Food Security” for EXPO 2015 in Milan (Italy), where it was presented by the writer on July 7th, 2015 (https://www.feedingknowledge.net/home/-/bsdp/5592/en_GB?_1_WAR_feeding_knowledgeportlet_redirect=%2F02-search%3Fp_auth%3D47l29FI2%26p_p_id%3D3_WAR_feeding_knowledgeportlet%26p_p_lifecycle%3D1%26p_p_state%3Dnormal%26p_p_mode%3Dview%26p_p_col_id%3Dcolumn-2%26p_p_col_count%3D2%26p_r_p_564233524_resetCur%3Dtrue%26).

In the specific context of Consorzio of Sannio Alifano, the overall results in terms of cost-benefit analysis, obtained comparing the irrigation season 2012 (pre-IRRISAT) and 2013 (post-IRRISAT), demonstrate water savings of about 18%, while a survey on a sample of monitored farms highlights peaks saving of about 25 to 30% without loss of production.

Since 2013, this system has been repeatedly refined and has been adopted not only in Campania as a whole, but also in irrigated regions of Austria and Australia. In these areas, the technology was rapidly transferred and applied with the same positive results.

In summary, the addition of a "water balance model" to the overall irrigational strategy allowed for additional positive developments in the management system of an irrigation network, by providing the water management body and users feedback to improve the results of water usage as well as saving water.

The overall logic process is illustrated by the flow chart shown in Figure 6.

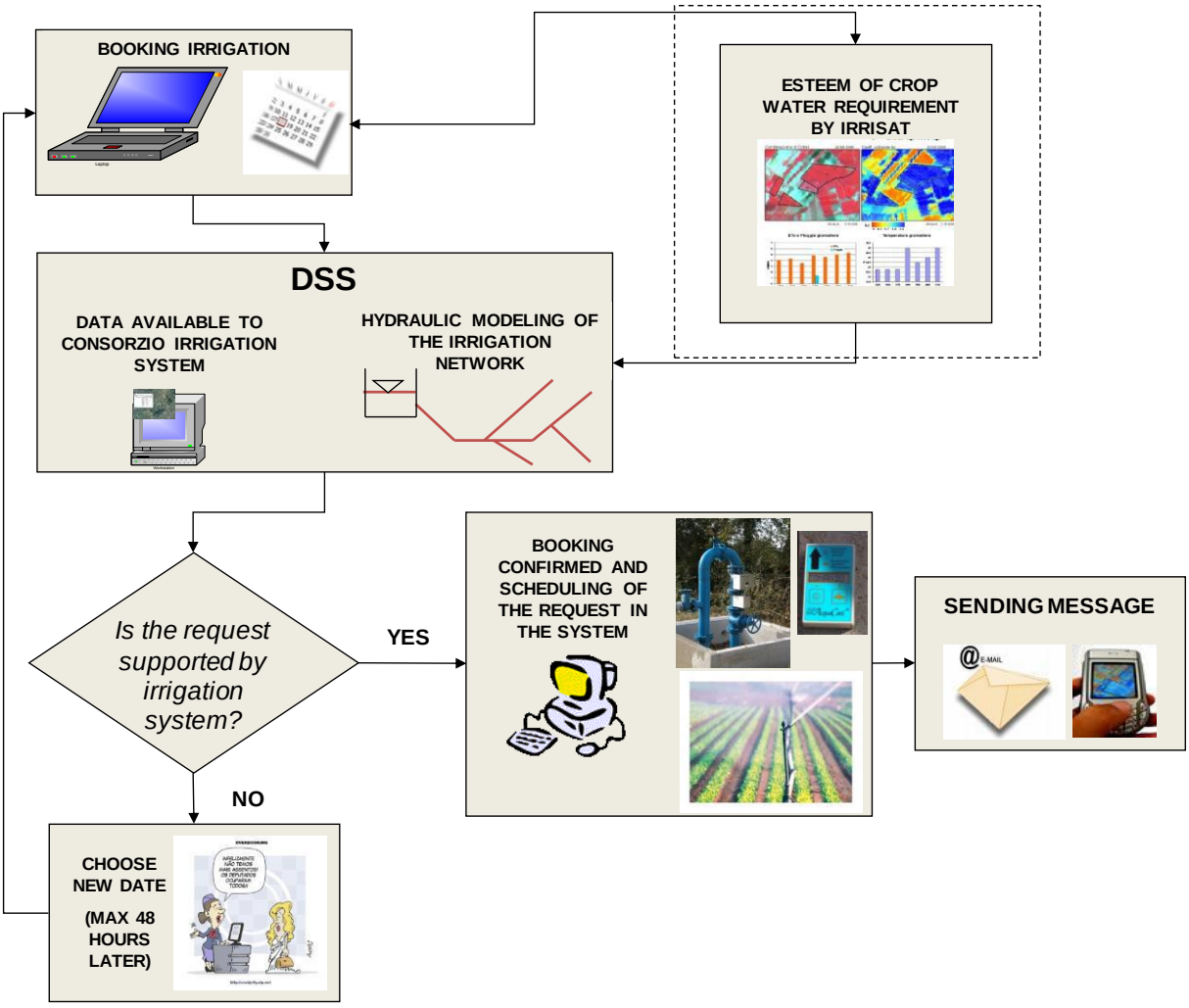

Fig. 6. Flowchart of the "water balance model" showing model input to the overall irrigation system.

Conclusions

Today the availability of computer programs dedicated to the management of network systems and databases, encourages, if not requires, managing bodies of water resources in agriculture to adopt decision support systems (DSS) for the management of irrigation networks. Current DSS are characterized by user-friendly interfaces. Within them, it is possible to implement water balance and crop growth models, geographic information systems and, most importantly, satellite and remote sensing data. Such systems enhance the ability of the managing body to conserve and effectively apply a vital natural resource in the production of food, water. With DDS in place within a water management structure, end users, for their part, only have to express their needs to the management body through a request for watering intervention. In doing so, as a result of a small waiver of absolute freedom of action (i.e., not being able to irrigate at times and volumes of their own choosing but in accordance with the water manager), the users reap the benefits of frequent consultation with experts of the managing body for the solution to their business needs, thereby gaining the ability to irrigate their fields with more certainty in terms of irrigation times and volumes, with the end of maximizing crop yields.

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STATISTICALLY-BASED DETECTION OF CLIMATE CHANGE

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Abstract

Climate change (CC) is the change in climate over time due to natural variability or human activities. Two aspects of the CC must be elucidated: on one hand, the detection of the CC, that is, the proof that the climate has actually changed by identifying statistically significant trends or breaks in the long-term climate data and, on the other hand, there is the problem of CC attribution that includes the identification, at a specified confidence level, of the most likely causes of the CC. Knowledge of trends and variations of current and historical climate variables is relevant to the sustainable management of water resources. One of the important research needs in the CC is to analyze and detect the historical changes in the climate system. We will present the main simple statistical methods for detecting trends in time series of climate data. However, we should, first, check the quality of climate data by verifying the homogeneity of the climate series using statistical tests such as the standard normal homogeneity test or Buishand's range test. Then, we should ensure that climate data satisfy the conditions for application of the methods of trend detection; among them, the independence of observations which is unlikely with climate data. The 'trend-free pre-whitening' statistical approach will be applied to eliminate autocorrelation in data. Finally, only after these two stages of exploratory data analysis, we can test for change detection or existence of trends. There are parametric and nonparametric methods. The former requires that data are normally distributed while the latter do not require any particular form of statistical distribution. The main methods will be presented with their conditions of application and will be illustrated with a concrete example of a series of climate data.
Keywords: *Autocorrelation, climate change, detection, homogeneity, trend*.

Introduction

The Intergovernmental Panel on Climate Change (IPCC) defines climate change (CC) as any change in the state of the climate that can be identified (by statistical tests) by changes in the mean and / or the variability of its properties and that persists for an extended period, typically decades or longer. The CC may be due to natural internal processes or external forcings related to human activity.

Detection of CC is the demonstration that the climate has changed by identifying statistically significant trends or jumps in long-term climate data. In contrast, the attribution of the CC includes the identification, at a given confidence level, of the most likely causes of such change. A key step in research on the CC is to be able to reveal that a change or a trend is present in historical climate records. The CC is detected when the observed overall average trend rises unequivocally above the background natural variability.

In trend analysis, one should, first of all, check the quality of climate data by verifying the homogeneity of the climate series by statistical tests. Then, we should ensure that climate data fulfill the conditions for application of trend detection methods. Among these conditions, there is the independence of observations. Finally, after these two stages of exploratory data analysis, we can test the change detection or the existence of trends by parametric or non-parametric methods.

Checking the homogeneity of climatic series

Breakpoints are discontinuity moments in a time series that can be caused by changes in observation locations, equipment, measurement techniques, environmental changes, etc. Inferences drawn from climate series frequently depend on 'continuity of the measurement process': lack of breakpoints. Chen and Gupta (2000) note that the linear trend estimates are reliable only when the series are consistent over time. Before studying the trends, the homogeneity of the series should be evaluated. Several statistical tests exist. We present two of them: the Buishand amplitude test and the standard normal homogeneity test.

Buishand cumulative deviations test

This test (Buishand, 1982), suggested by the World Meteorological Organization (WMO), is based on cumulative deviations from the mean:

$$S_k = \sum_{i=1}^{k} (Y_i - \overline{Y}) \qquad k = 1, K, n \tag{1}$$

where $\overline{Y}$ is the mean of the values $\mathbf{Y_i}$ and **n** is the number of these values.

For a homogeneous series of recordings, values of Sk fluctuate around 0. If Sk shows a maximum (minimum), a positive (negative) break is present. Rescaled adjusted partial sums Sk* are determined by dividing the Sk values by the standard deviation of the sample:

$$S_k^* = \frac{S_k}{D_y} \qquad k = 1, k, n \tag{2}$$

where $\mathbf{D_y}$ is the standard deviation of the sample.

Based on $\mathbf{S_k}^*$, a statistic ***Q***, sensitive to differences of homogeneity, is defined as:

$$Q = \max_{1 \le k \le n} |S_k^*| \tag{3}$$

If the magnitude of $\boldsymbol{Q}/\sqrt{\boldsymbol{n}}$ exceeds 1.30 then temporal series is heterogeneous (Yu et al, 2006).

Standard normal homogeneity test ***(SNHT)***

SNHT (Alexandersson and Moberg, 1997) is based on the statistic **T(k)** which compares the mean of the **k** 1st observations with that of the **n-k** remaining observations:

$$T(k) = k{z_1}^2 + (n-k){z_1}^2 \quad k = 1, \ldots, n \tag{4}$$

with

$$z_1 = \frac{1}{k} \sum_{i=1}^{k} \frac{x_i - \mu_x}{\sigma_X},$$

$$z_2 = \frac{1}{n-k} \sum_{i=k+1}^{n} \frac{x_i - \mu_x}{\sigma_X} \tag{5}$$

The break occurs when **T(k)** is located near the observation that maximizes the T variable and the null hypothesis of homogeneity is rejected if the test statistic $T_0 = max(T(k))$ exceeds the critical threshold. For a significance level of 5%, this threshold is 9.17 (Khaliq and Ouarda, 2007).

Conditions of application of trend detection methods

The parametric and non-parametric tests for trend require the observations forming the time series are independent and identically distributed (iid). In addition, parametric tests require that observations are distributed according to the normal law. Therefore, before making the trend tests, we should ascertain the normal distribution of data and the independence of observations (no autocorrelation).

Normal distribution

The normality of a series of observations can be verified either graphically or by formal statistical tests. The most commonly used graphs are the histogram and the quantile-quantile graph. Regarding statistical tests, there is a large choice: test of skewness and kurtosis coefficients, the Kolmogorov-Smirnov test, the Anderson-Darling test, etc. The most commonly used tests in climatology are the Shapiro-Wilk test and the median test. Time series will be considered to be coming from a standard normal distribution if at least one of the following criteria is met (Wu et al, 2007): the Shapiro-Wilk statistics ≥ 0.96 or the $|\text{median}| \leq 0.05$. In this case, the parametric tests are recommended. Otherwise, it is required to use non-parametric tests.

Independence of observations

In time series analysis, it is essential to consider the autocorrelation, defined as the correlation of a variable with itself over successive time intervals, before testing for trends. The application of non-parametric tests for the detection of trends can significantly affect the results. Therefore, the time series data must first be tested for the presence of one order autocorrelation coefficient (r1) at the 5% level, using a two-tailed test:

$$r_1 = \frac{\sum_{i=1}^{n-1}\left(X_i - \overline{X}\right)\left(X_{i+1} - \overline{X}\right)}{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2} \quad (6)$$

The value of the autocorrelation coefficient (r1) is tested against the null hypothesis at the 95% confidence interval, using a two-tailed test:

$$r_1\,(95\%) = \frac{-1 \pm 1.96\sqrt{(n-2)}}{n-1} \quad (7)$$

If r1 falls between the lower and upper limits of the confidence interval, the data are considered to be serially correlated and methods of pre-whitening, correction variance and trend-free pre-whitening should been used before analyzing for the trend.

Change detection or existence of trends

The objective of the trend test is to determine whether the values of a random variable generally increase or decrease, in statistical terms, over a time period. Parametric or nonparametric tests can be used to decide whether there is a statistically significant trend. The steps of the analysis are: (1) test of the effect of serial correlation (autocorrelation), (2) detection of the trend by applying the Mann-Kendall test, the Spearman test and the linear trend method and (3) estimation of the magnitude of the trend using the Sen estimator. Different methods of statistical tests are used to detect trends in climate time series; these are classified as parametric or non-parametric (Dahmen et al, 1990). Parametric tests are more powerful but require that the data be independent and normally distributed, which is rarely the case for climate data. For non-parametric tests, the data must be independent, but outliers are better tolerated.

Parametric methods

Student t test of the Pearson correlation

The classical Student *t* test assesses the significance of the correlation between the values of meteorological variables and their observation times. The Pearson correlation coefficient is calculated from the covariance and standard deviations of the two variables. The Student *t* test is then used to test p-value of the test statistic.

Linear trend model

Given a temporal series $X_1, X_2, \dots, X_n$, then the linear trend model is given by:

$$X_t = \beta_0 + \beta_1 t + \varepsilon_t, t = 1 \dots n \quad (8)$$

where β_0 and β_1 are the intercept and the slope, respectively and $\{\varepsilon_t\}$ are independent random errors and normally distributed with a zero mean and a constant variance σ^2.

This model is adjusted using the simple linear regression approach. A table of analysis of variance with the p values corresponding to the intercept and slope are provided. The p value of the slope is often used to make a two-tailed test for the significant trend in the time series. This parametric test is based on the t-distribution and requires several assumptions as above. This test is also sensitive to the presence of outliers.

Most studies of climate trends assume a priori that these trends are linear and use, therefore, the least squares linear fit to extract this trend (IPCC, 2013). The analysis of the linear trend can quantify the trend in a simple way. However, estimating the climatic trend using the linear approach is not physically realistic and can lead to uncertainty because the time behavior is complex in a changing climate.

Non-parametric methods

The most common non-parametric tests are the Mann-Kendall (Kendall, 1975) and the test of the Spearman rank correlation (Sneyers, 1990). Mann-Kendall test is the most commonly used. Less common, the Spearman test was used to detect monotonic trends in climate data.

Mann-Kendall test

This test has been widely used to test the randomness versus trend. This is a procedure based on the ranks, robust to the influence of extreme and suitable for asymmetric variables. The null hypothesis, H_0, stipulates that the seasonally adjusted data $\boldsymbol{x_1, \dots, x_n}$ are a sample of $\boldsymbol{n}$ iid random variables. The alternative hypothesis, H_1, of a two-tailed test is that the distributions of $\boldsymbol{x_k}$ and $\boldsymbol{x_j}$ are not identical for all $\boldsymbol{k, j<n}$ with $\boldsymbol{k=j}$. The statistics of the S test, with an average 0 and variance calculated by the equation (11), is calculated using equations (9) and (10) and is asymptotically normal:

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} \operatorname{sgn}(x_j - x_k) \quad (9)$$

$$\operatorname{sgn}(x_j - x_k) = \begin{cases} +1 \text{ if } (x_j - x_k) > 0 \\ 0 \text{ if } (x_j - x_k) = 0 \\ -1 \text{ if } (x_j - x_k) < 0 \end{cases} \quad (10)$$

$$Var(S) = \left[\frac{n(n-1)(2n+5) - \sum_t t(t-1)(2t+5)}{18} \right] \quad (11)$$

The notation t is the importance (extent) of any given tie and denotes summation over all ties. If the sample size n>10, the standard normal variable z is calculated using equation (12):

$$Z = \begin{cases} \frac{S-1}{\sqrt{Var(S)}} & \text{if } S>0 \\ 0 & \text{if } S=0 \\ \frac{S+1}{\sqrt{Var(S)}} & \text{if } S<0 \end{cases} \quad (12)$$

In a bilateral test for trend, H_0 is accepted if $|z| \leq z_{1-\alpha/2}$ at the significance level α. A positive value of S is a increasing trend and the negative means a decreasing trend.

Spearman rank test

This test is the nonparametric analogue of the *t* test of Pearson correlation. The statistic of the test is the rank correlation coefficient of Spearman r_s, which is the correlation between the ranks of meteorological variables and observation times. Because of the use of ranks instead of the observed values, the sampling distribution of r_s for a stationary process can be calculated without knowledge of the distribution function.

It is a non-parametric method, based on the ranks. In this test, which assumes that the time series data are independent and identically distributed, the null hypothesis, H0, shows no trend over time; the alternative hypothesis, H1, is that there is a trend and that the data increase or decrease with time i (Yue et al, 2002). The statistics of the R_{sp} test and the standardized statistics Z_{sp} are:

$$R_{sp} = 1 - \frac{6\sum_{i=1}^{n}(D_i - i)^2}{n(n^2-1)} \quad (13)$$

$$Z_{sp} = R_{sp}\sqrt{\frac{n-2}{1-R_{sp}^2}} \quad (14)$$

D_i being the rank of the i^{th} observation, ***i*** the chronological order number, ***n*** the total length of the time series, and Z_{sp} is the value of the Student ***t*** distribution with (***n***–2) degrees of freedom. Positive values of Z_{sp} represent an increasing trend while negative values represent decreasing trend. The critical value of ***t*** at the signification level of 5% from the Student distribution table is defined as ***t***(***n***-2, 1-***α***/2) (Dahmen and Hall, 1990). If $|Z_{sp}| > t(n-2, 1-\alpha/2)$, H_0 is rejected and a significant trend exists in the time series.

Sen's estimator of slope

The importance of the trend is estimated with the help of the Sen's estimator. In this case, a linear trend is present and thus the true slope is estimated by this method. Here, the slope (T_i) for all pairs of data is first calculated as (Sen, 1968):

$$T_i = \frac{x_j - x_k}{j-k} \text{ for i = 1,2,.....,N} \quad (15)$$

where x_k and x_j are represented as data values at times ***j*** and ***k*** (***j***>***k***), respectively. The median of these ***N*** values of T_i is considered as the Sen's estimator of the slope which is given as:

$$Qi = \begin{cases} T_{\frac{N+1}{2}} & N \text{ is odd} \\ \frac{1}{2}\left(T_{\frac{N}{2}} + T_{\frac{N+2}{2}}\right) & N \text{ is even} \end{cases} \quad (16)$$

The Sen's estimator is computed as $Q_i = T^*(N + 1)/2$ if ***N*** is odd and as $Q_i=[T_{N/2}+T_{(N+2)/2}]/2$ if ***N*** is even. Finally, Q_i is estimated by a bilateral test at the confidence interval of **100*(1 - *α*)%** and thus a true slope may be derived from the non-parametric test Q_i with a positive value of Q_i indicating an increasing trend and a negative value meaning a decreasing trend.

Conclusions and recommendations

The research on historical changes in the climate system is a necessary component of research on CC because the historical climate records provide context on natural variation in order to

detect the CC forced by anthropogenic activity. A reliable study of the CC requires, beforehand, climatic series of quality that could be identified by statistical tests of homogeneity. Then, one should ensure the absence of autocorrelation in the climatic series; otherwise, we should manipulate these series to eliminate this serial correlation using, for example, the pre-whitening or variance correction approaches. The next step is to test the normality of the climate series; in the affirmative, trend analysis will be performed by the use of parametric tests such as Pearson correlation and linear regression. If, in contrast, the normality condition is not met, we should use the non-parametric tests such as the Spearman rank correlation, the Mann-Kendall test, or the Sen's estimator of slope.

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HOUSEHOLD FOOD WASTE IN MOROCCO: AN EXPLORATORY SURVEY

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Abstract

Food losses and waste is generated in important amounts across the food chain, from production to consumption, imposing serious environmental, social and economic costs. More attention is paid in Morocco to food losses while food waste (FW) is generally overlooked. There are few research activities on this problem in the kingdom. This exploratory study aims to evaluate household FW in Morocco. An online survey with 122 Moroccan households was conducted in February-May 2015 to assess the knowledge and relative importance of FW; attitudes towards FW; impacts of behaviors regarding food and food management; quantity and value; as well as barriers and willingness to behavioral change. Sample is gender-balanced (52% female and 48% male) and rather young (80% are less than 44 years old) while most of respondents have high education level. Results show that household's planning and shopping activities are important predictors of FW. On the other hand, attitudes may change according to periods especially in Ramadan (87% declare that FW is higher during this month) and also to the category of food (most wasted product groups are cereals and bakery products, fruits and vegetables). Most of the respondents have a good understanding of food labels. It seems that FW is widespread in Morocco as only 3.3% of respondents declare that they do not waste any food. About two-fifths (39%) declare that their households throw away at least 250 g of still consumable food each week. The economic value of FW generated each month is more than 60 Moroccan Dirham (≈ 6 US$) for 54% of respondents' households. In order to change consumers and household's FW behavior, efforts should be directed towards providing consumers with skills and tools to deal with their food-related activities and to consider environmental and economic impacts of food waste.

Keywords: *Food waste, Online survey, Household behavior, Morocco*

Introduction

Reducing food losses and waste (FLW) is attracting growing public attention at the international, regional and national levels, and is widely acknowledged to contribute to abating interlinked sustainability challenges such as food security, climate change, and water shortage. However, the pattern and scale of food waste throughout the supply chain remains poorly understood, despite growing media coverage and public concerns in recent years (Bagherzadeh et al., 2014). FLW is generated in important amounts across the food chain, from production to consumption, imposing serious environmental, social and economic costs (Gustavsson et al., 2011).

FLW is also an issue in Mediterranean countries. In the final declaration of the 10th meeting of the CIHEAM member states' agriculture ministers, held in Algiers in February 2014, the

relevance of food waste issue in the Mediterranean countries was strongly stressed (CIHEAM, 2014). The lack of data concerning the Southern and Eastern Mediterranean Countries means that research and cooperation on this matter need to be improved (Charalampopoulos et al., 2014).

Regarding Morocco, more attention is paid to food losses while food waste (FW) is generally overlooked. There is a need of research to bridge the knowledge gap on this problem. However, some recent initiatives have been promoted by some public institutions to address this important issue in the kingdom. For instance, on June 11th 2015, the Moroccan Ministry of Agriculture and Fisheries in partnership with the Food and Agriculture Organization of the United Nations (FAO) launched a strategic project in order "*to develop a national strategy and an action plan to reduce FLW in Morocco*". The main activity consists on a study on FLW in the country for a selected key food supply chains. The study aims to develop a vision and strategic directions that will conduct into an action plan to reduce FLW by 50 % by 2024 (Moroccan Ministry of Agriculture and Fisheries, 2015).

This current exploratory survey anticipated the abovementioned study and aims to evaluate household FW in Morocco. The specific objectives concern knowledge and perceived relative importance of FW; attitudes towards food waste; impacts of behaviors regarding food and food management on food wastage; quantity and value of food wasted; and barriers and willingness to behavioral change.

Materials and Methods

The present paper is based on the results of a voluntary survey in Morocco using a questionnaire that was adapted to Moroccan context from previous questionnaires and studies on FW carried out by the Office of Environment and Heritage in 2011 in the State of New South Wales (NSW), Australia (OEH, 2011), and by the University of Bologna (Last Minute Market, 2014).

The tool used to conduct the survey is a self-administered questionnaire. It was made available in English, French and Arabic languages from February till the end of May 2015 through the Survio website (www.survio.com). Participation was entirely volunteer bases and responses were analyzed only in aggregate.

The questionnaire consisted of 26 one option and multiple choice questions structured in 6 sections:

food purchase behavior and household food expenditure estimation; knowledge of food labeling information; attitudes towards food waste; extent of household food waste; economic value of household food waste and willingness and information needs to reduce food waste. In the introductory part of the questionnaire the concept of FLW was introduced to inform the respondents.

Various institutional communication channels were used to disseminate the survey, such as: institution website (National school of Agriculture of Meknes); professional forums (https://fr.groups.yahoo.com/neo/groups/tech_agro/info); social media: (https://www.facebook.com/groups/aniphop) and mailing lists.

Data were analyzed using descriptive statistics (e.g. means, max, min), in order to get a general picture of frequencies of variables, using Microsoft Excel.

Out of 129 questionnaires received, 7 were not considered because there were missing data. Therefore, the total number of the sample is 122 adult Moroccans. The sample is almost gender-balanced (52% female and 48% male), rather young (80% are less than 44 years old) and most of the respondents (more than 69%) have high education level. More than half of the respondents are married with children (55.7 %). As for household composition, half of the respondents lives in households with up to 3 members, while the rest of the sample with 4 to 6 members at home.

Results and Discussion

The survey showed that respondents use to buy food products in different *shopping places* but more from small and minimarkets and rarely on farm. In fact, the market or what is called "souk" (traditional market place in Morocco) takes the first rank with 37%. This could be due to the fact that most consumers look for good quality products with low price. Another point that would be highlighted is that the fact of visiting the souk (mainly the weekly one) is considered as an inherited custom since a large number of Moroccans goes to the traditional market place not only to do shopping but also to meet friends and relatives. At the second rank, come minimarkets with 34%. Actually, one or two minimarkets could be found at each living hood street mainly in big cities of the country. Therefore, the proximity as well as the long working days (i.e. lack of time) for many consumers encourage them to purchase the foodstuff from the closest minimarket. During the last decades, the number of hypermarkets has increased and a competition on presenting different deals begins to get underway, which gives more confidence to consumer to go through. The wide range of available food products at the same location would be also a positive feature that persuades consumers to choose this shopping. Services, which are better on supermarkets than in other shopping places, would be a further reason for resorting at supermarkets. Only 2% of the respondents opt for the farm as a place of food shopping. It is an unusual habit of shopping in Morocco mainly for people from big cities. Furthermore, the supply chain is not well organized, which make direct sale by farmers quite complicated. Only in few places in the kingdom, fresh fruit and veggies start to be sold on the farm.

Regarding *food shopping frequency*, most of the respondents purchase food once a week (31%) or twice per week (25%) but only 15% respondents do their food shopping every day. This frequency could be related to the working and living conditions of every respondent. In addition, this frequency is highly related to the place of food shopping as discussed before.

The survey results highlighted that *the food budget* exceeds the level of 900 Moroccan dirham, MAD (10 MAD ≈ 1US $) per month in more than 65% of the cases. This category corresponds probably to households composed of 4-6 members and more. However, for the purpose of the present research, rather than considering the total food budget, it makes more sense to focus on the percentage of food budget that is lost when food is wasted.

The use of *shopping list* does not appear as a usual technique for most interviewees (30%). Only 25% of the respondents use always a list for purchasing food. The remaining people (45%) resort sometimes to using the list depending on shopping purpose. Consequently, there is a greater awareness that the use of a list can make shopping smarter, and hence contribute, likely, to a reduction of food waste.

Regarding *attraction to offers*, an important number of respondents are always interested (34%) or sometimes (42%) by good deals. The influence of these offers would have sometimes a great impact on the purchased quantity of food. This happens very often in Morocco during some specific periods of the year (Ramadan, religious holidays and other occasions). Therefore, a large part of the purchased foods will eventually have to be stored. This would increase the probability of throwing food thereafter. Much attention is thus to be paid on food labeling predominately the expiry date.

Concerning "*use by*" food label*;* only 85% of respondents understand and have knowledge about this label as they think that food should be consumed or discarded by this date. While 13% consider that the food is still safe to eat after that date if it is not damaged or spoiled. In the case of "*best before*" label, it is surprising that 68% of respondents confuse this label with "*use by*" one as they think that food should be discarded after this date. Only 31% of the survey sample showed good understanding of the meaning of this label. This shows that there is a difficulty in understanding of the two label dates by Moroccan consumers and clear

confusion between the two. Furthermore, it should be pointed out that “best before” label is not widespread in Morocco, and, consequently, not well known by Moroccan consumers.
Luckily, most respondents (88.5%) expressed a high *concern on food waste* and the attitude to avoid it as much as possible. This could be due to the fact that the Moroccan culture, customs and traditions, which are dominated by a religious character, make the act of throwing food something outrageous. About 7% of them are aware of the problems associated with food waste, but they do not think they will change their behavior in the near future. Nevertheless, a very low percentage (4.5%) of them considers FW as a non-relevant problem.
Food waste amount changes from a *period of the year* to another. In fact, according to 87 % of respondents FW increases during the fasting month of Ramadan. This is due to increased difficulty of planning meals during this month. In fact, many households in Morocco use to cook more than what they can eat for fast breaking. Therefore, this highlights the importance of organizing awareness rising campaigns before and during the month of Ramadan in order to reduce FW.
Concerning of the *quantity of food waste*, 50% of the respondents estimated that a very small amount of food is wasted in their household in comparison with 20% of them who consider that they waste much more than it should not. A quarter of them assume that the food waste quantity at the household is reasonable. This result shows that Moroccan consumers are, in general, aware of FW issue. In fact, only 3% of them considered that no food is wasted in their household.
The *uneaten food* is managed in different ways by respondents’ households. Sometimes, the same household uses different uneaten food management strategies. The study showed that, unfortunately, 69% of respondents throw the uneaten food in the trash. However, an important part of respondents (24%) manage uneaten food in good way by giving it as donation. Meanwhile, some respondents use uneaten food to feed animals (24%) or to produce compost (2.5%). While, these two last uneaten food management strategies are clearly better than throwing it in the trash, they do not represent anyway good strategies for FW reduction as also food fed to animals or used to produce compost is to be considered as wasted food.
The frequency of throwing away *leftovers* or *food considered as not good* has been also pointed out in the survey. The results showed that only 12% of the respondents don’t throw leftovers in comparison with more than 46% of them who declared throwing food for less than one time a week. On the other hand, more than 26% of the respondents throw food leftovers 1 to 2 times a week. However, 15 % throw away food leftovers even more than 2 times a week. To sum it up, a high percentage of respondents (88%) throw at least once per week leftovers or food considered not good.
Different *actions towards food* undertaken by the respondents affect the amount and extent of household FW. Among the actions that likely have a negative effect on FW can be mentioned using the leftover and the remaining food as well as eating outdoor. In fact, the survey result showed that 92% of respondents consume at least a meal remaining day before. This practice is certainly good for reducing FW. The survey showed also that 80% of respondents eat out or eat a takeaway at least once a week. This action can reduce also household FW but not necessarily FW in general. In fact, FW can be generated in this case also in food services (i.e. restaurant) that show the importance of involving food services in FW reduction strategies and initiatives. The percentage of respondents of people eating outdoor is quite high taking in consideration the Moroccan context as the Moroccan society still has a traditional and conservative character thus preferring to eat, especially mean meals, at home. This high percentage may not be representative, for the Moroccan context, as the sample considered in this survey is young and has a high education level; so that respondents can be highly influenced by western food habit and consumption pattern.

The result of the survey show that the *main reason behind waste of food* is due to bad food management at home so under the responsibility of final consumer. As the matter of fact, according to 67% of respondents, foods are wasted because they are left in the fridge for a long time. In this case, food is throwing away because it has bad smell or taste, has mold, and does not look good or expired. Poor conservation is a factor determining the increase of FW also for foods that are not stored in the fridge. Bad food management at home, such as errors in meal planning (30%), implies that there are often remains of cooked dishes and that portions are too heavy. Therefore, it is crucial to organize communication campaigns, especially through mass media (Radio, TV), on food management with a particular reference to good practices and rules regarding food storage and preservation at home. Reasons that are due to the food industry and food retail mentioned by respondents are that labels generate confusion and the package does not have always good size.
The *quantity of thrown food* per week depends on different factors inculing houshold composition. About the half of respondents (45%), declared that they never throw food which is still consumable, while (39%) of respondents affirm that their households throw away at least 250g of still consumable food each week. It is worth highlighting that according to the survey results, 55% of respondents waste food even if the quantity wasted is generally small. Hence, urgent measures are needed to address the issue of FW in Morocco as FW is also considered as a waste of resources (land, water, and energy) as well as money.

Table 1: Quantity of thrown food per household and per week

Answer choices	**Ratio (**%)
I do not throw food that is still consumable	45.1
Less than 250g	15.6
Between 250 and 500 gr	21.3
Between 500 gr and 1 kg	8.2
Between 1 kg and 2 kg	5.7
More than 2 kg	4.1

Source: Authors’ elaboration based on the survey results.

The extent of FW varies from a *food group* to another. In general, perishable products are the most wasted food. This result is in line with the outcomes of FAO’s promoted study on global FLW (Gustavsson et al., 2011). In particular, the survey showed that the most wasted food group in the Moroccan context is cereals and the bakery products, followed by fruits, vegetables. Meanwhile, the least wasted food group is roots and tubers, dried vegetables and oilseeds, meat and meat products, and fish and sea food. Milk and milk products are moderately wasted. The high percentage of cereal and bakery products that is wasted is a big environmental and economic problem considering the high amount of these products, especially consumed by Moroccans. This is also a political issue in Morocco, as bread is subsidized in the Cherifian kingdom, so bread waste is considered also as a waste of public budget and, consequently tax payers’ money. It is interesting to notice that, not only *perishability*, but also price is an important factor in determining the extent FW. In fact, meat and meat products, and fish and sea food that are also perishable products, but quite expensive, are among the least wasted foods.

Table 2: Ratio of thrown food per group

Food waste ratio / Food groups	**Less than 2%**	**3 to 5%**	**6 to 10%**	**11 to 20%**	**More than 20%**
Cereals and bakery products (bread, rice, pasta, etc.)	33.6	20.5	13.1	13.9	18.9
Roots and tubers (potatoes, etc.)	63.1	18.0	11.5	2.5	4.9
Dried vegetables and oilseeds (e.g., peas, chickpeas, olives, sunflower)	78.7	12.3	4.9		0.8
Fruits	49.2	27.0	15.6	6.6	1.6
Vegetables	32.8	40.2	16.4	4.9	5.7
Meat and meat products	78.7	11.5	8.2	1.6	0
Fish and seafood	85.2	9.0	4.1	1.6	0
Milk and milk products	59.0	23.8	9.0	5.7	2.5

Source: Authors' elaboration based on the survey results.

Economic value of household food waste depends not only on waste amount (so also on household composition), and the composition of FW, but also on household food habits and consumption patterns. According to the survey, the value of household FW generated each month is more than 60 Moroccan dirham for 54% of respondents' households. The weighted average economic value of the monthly FW generated by each responding Moroccan household is about 100 MAD. This value represents of around 13% of the weighted food budget for the considered sample.

Table 3: Economic value of food waste generated each month by household

Answer choices	**Ratio (**%)
Less than 5 US$	45.9
Between 6 and 20 US$	42.6
Between 21 and 50 US$	10.7
More than 51 US$	0.8

Source: Authors' elaboration based on the survey results.

Better information is the key to reduce household FW. In fact, among the necessary *conditions to waste less food* mentioned by responds *are to be* better informed about the negative impacts of food waste on the environment (44%) and on the economy (38%). Food industry and food retail can play an important role in food waste reduction by improving packaging and making food labeling clear. Government actions to reduce FW can include introducing a new taxing system penalizing food wastage making it inconvenient from the economic point of view. However, implementing such a system is not an easy task in Morocco, giving the inefficient waste management system.

According to the exploratory survey, *information* about different issues are needed, sometimes even simultaneously, by respondents for reducing food waste such as advices on how to properly store food (59%), organizations and initiatives that address the prevention and reduction of food waste (49%), recipes prepared with leftovers (38%). Also

information about how to assess the freshness of products is needed by about a third of respondents (35%).

Conclusion

Food-related behavior and attitude are important factors in determining the amount and extent of food waste. Food habits change according to year's periods, as during the fasting month of Ramadan, with implications in terms of FW. However, the amount of household FW is affected not only by food habit and behavior, but also depends on food groups. In fact, most wasted foods are cereals and bakery products. This fact is alarming taking into consideration that these are the most consumed products in Morocco. It seems that there is still some confusion regarding food labels, which increases the amount of FW. This result represents a big problem as our sample has good education, so it is legitimate to assume that the situation is worse especially considering the relatively high percentage of illiterates in the kingdom. The estimated economic value of FW is rather low but still a source of concern taking into consideration its share in the household food budget. In order to reduce household FW, effort should be directed towards changing consumer behaviors and habits especially household management practices (i.e. fridge management), by providing consumers with knowledge and information to deal with their food-related actions, while fully considering economic and environmental impacts of food waste. The recent initiative of the Moroccan Ministry of Agriculture - in collaboration with FAO - is a step in the right direction, but it is not sufficient. There is a high need for more initiatives (targeting not only FW reduction, but also FW prevention) especially awareness rising campaigns. For that a multi-stakeholder approach and active involvement of all key actors of the food chain, including the private sector as well as civil society, is highly recommended in the future to effectively, efficiently, and sustainably address the issue of FLW in Morocco. This should be put high on the government agenda taking into consideration the environment and economic implication of FLW.

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ENVIRONMENTAL IMPACTS OF STANDARD OF LIVING: A CASE STUDY OF MARDAN DISTRICT, KHYBER PAKHTUNKHWA IN PAKISTAN

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Abstract

Household income plays an important role in the improvement of the standard of living. However as the standard of living of the household improves, it also causes environmental degradation. This study investigates the environmental impacts of living standard of the households in the Mardan District. The impact of other factors such as household size, location of the household (urban or rural) and education of the households is also estimated. The study used primary data of 267 households collected through a questionnaire. The sample has been proportionally allocated between two sub-districts namely, Mardan and Takhtabhai. Accordingly the sample size for each Tehsil is 193 and 74 households respectively. Further, the sample size for each Tehsil is allocated to rural and urban areas proportionally. Accordingly, from the whole Mardan districts, 54 households from urban and 213 households from rural areas were selected. The respondents in both rural and urban areas have been selected randomly. The analysis was made through descriptive statistics and regression model. Findings show that household income is significant factor in increasing CO_2 emissions in Mardan District. A positive relationship is also found between household size, education and CO_2 emissions. Findings also revealed that Environmental Kuznets Curve (EKC) is valid in case of rural areas but not in urban areas because the urban consumers use more carbon intensive commodities. Hence an effective policy formulation stressing on public awareness programs, incentives of tax reductions, and better public transport network is conducive to the reduction of CO_2 emissions in the selected District.

Keywords: *Standard of living, Environmental impacts, Environmental Kuznets Curve, Household awareness, Emission factors*

Introduction

The standard of the living is the degree of wealth available to an individual or family with which they can satisfy their wants/consumption of goods and services necessary for well-being. There are many factors that influence the standard of living of an individual or family. The higher the income, the higher is the standard of living because people will be able to buy more goods and services (Perry, 2013). Improvement in the standard of living always remained the priority of the governments but it is also well known fact that improvement in the standard of living or income of the people the usage of vehicles, refrigerators, air conditioners and other fuel items and electrical appliances increases, which ultimately adds to the carbon dioxide emission in the economy. Similarly, household income, urbanization, age of the main income provider and type of the house are the strongly influencing factors of CO_2 emissions (Baiocchi, Minx, and Hubacek (2010); Li and Wang (2010). Similarly, as the food expenditures increases, the solid wastes also tend to increase (Feng, Zou, and Wei (2011).

The GDP per capita increased in Pakistan from US $631 in the year 2004 to US $1024 in the year 2010 (World Bank, 2014) but on the other hand the mean estimated annual cost of environmental and natural resource damage is about 365 billion Rs. per year or 6 percent of GDP. Also, the per capita emissions have been increased from 0.84 metric tons per capita in the year 2004 to 0.93 metric tons per capita in the year 2010 (World Bank, 2014).

Mardan is a typical district of Pakistan where, in the year 2013, about 98% of the household are using electricity for lighting without having much rural urban variation. The usage of gas/oil for lighting in rural and urban areas is 1% and 2% respectively. Similarly, the usage of gas as fuel in urban and rural areas in the year 2013 was 92% and 18% respectively while wood/charcoal usage was 7% and 56% respectively (Government of Pakistan, 2014). In Khyber Pakhtunkhwa province, the per capita consumption of gas in year 2010 was 1422 Cubic Meters but has grown to 2494 cubic meters in the year 2013 (Government of Khyber Pakhtunkhwa, 2014). In the year 2006-07, 65% of the household used gas/oil for cooking in urban areas of District Mardan (Government of Pakistan, 2007) while in the year 2012-13, it increased to 92% (Government of Pakistan, 2014). Similarly in rural areas, 9% of the household used gas/oil for cooking in the year 2006-07 (Government of Pakistan, 2007) , increasing to 18% in the year 2012-13 (Government of Pakistan, 2014). In this District, the total population of registered motor vehicles was 115814 in the year 2012 (Government of Khyber Pakhtunkhwa, 2014) while this was 98576 in the year 2011 (Government of Khyber Pakhtunkhwa, 2012). Apart from these the population of the District also increased significantly over the years. Based on these facts, this study looks how much environmental degradation (CO_2 emissions) takes place followed by estimating the impact of income and non-income factors on carbon dioxide emissions at household level in rural and urban areas of District Mardan.

The literature is rich about the relationship between economic growth and environmental degradation based on secondary data such as (Grossman & Krueger, 1991) who also proposed the idea of environmental Kuznets curve (EKC) the first time. The same was also concluded by Hung and Shaw (2004). Some studies validated the EKC hypothesis for some countries such as Ubaidillah (2011) and (Uddin, 2014). While some didn't validate the hypothesis of EKC such as Mrabet, Achairi, and Ellouze (2013) and Akpan and Chuku (2011). The validity of EKC is not similar for each environmental quality while varies across environmental indicators. Ni, Lu, Lan, Gao, and Pan (2010) found that the indicators of surface water mostly support the EKC, while ambient air and near-shore water's indicators do not support it. Similarly, Shaw, Pang, Lin, and Hung (2010) investigated that EKC hypothesis confirmed by SO_2 and total suspended particulates while it does not confirmed in the case of NO_X. The present study advances to literature through estimating the CO_2 emissions using emission factors followed by estimating its determinants at household level in rural-urban areas of District Mardan.

Materials and Methods

To assess the impact of the standard of living on the environment the present study is based on primary data that have been collected through a questionnaire. The household has been selected randomly for getting information. The questionnaire consists of the information relevant to the household's energy consumption in the form of electricity, natural gas, firewood, liquefied petroleum gas (LPG) cylinder, electrical appliances (refrigerators, freezers, air conditioners, air coolers, fans, washing machine, bulbs, energy savers, electric heater, geyser, iron, vaccume cleaner, television, desktop computer, laptop computer and microwave oven), fuel (petrol and compressed natural gas) used in personal vehicles (car and motorcycle), wastes produced by the household and the household characteristics including household income, family size, location (urban or rural) and education of the main income provider of the household.

The total estimated number of the households during the year 2014 was 330544 (Government of Khyber Pakhtunkhwa, 2014). Keeping the confidence level as 95 percent and confidence interval at ±6 percent, the required sample size calculated was 267 households. The sample has been proportionally allocated between two sub-districts namely, Mardan and Takhtabhai.

Accordingly the sample size for each Tehsil is 193 and 74 households respectively. Further, the sample size for each Tehsil is allocated to rural and urban areas proportionally. Accordingly, the calculated sample size for urban and rural areas of Tehsil Mardan is 45 and 148 households, respectively. The sample for urban and rural areas of Tehsil Takhtabhai is 9 and 65 households respectively. The respondents in both rural and urban areas have been selected randomly.

The study used household income as an indicator for the standard of living as proposed by Government of New Zealand (2007), OECD (2013) and Perry (2013). Apart from income, the other variables included are the household size, location of the household (urban or rural) and the education of the main income provider of the household. The household consumption has been converted into carbon emissions through emission factors proposed by DEFRA (2012), Carbon Neutral Company (2014), Intergovernmental Panel on Climate Change's (IPCC's) Guidelines for National Greenhouse Gas Inventories (Intergovernmental Panel on Climate Change 2006). This procedure has also been used by Zhao and Cui (2013) and Kavi and Viswanathan (2013).

The conversion formula is given as:

$$CO^{hh}{}_{2e} = \Sigma C_i * EF_i$$

Where,

$CO^{hh}{}_{2e}$ = The household carbon dioxide emissions

C_i = The household consumption of the ith energy in the form of electricity, natural gas, firewood, liquefied petroleum gas (LPG) cylinder, electrical appliances (refrigerators, freezers, air conditioners, air coolers, fans, washing machine, bulbs, energy savers, electric heater, geyser, iron, vaccume leaner, television, desktop computer, laptop computer and microwave oven), fuel (petrol and compressed natural gas) used in personal vehicles (car and motorcycle) and wastes produced by the household.

And

EF_i = The CO_2 emission factors of the ith energy

The emission factors for electricity, natural gas, waste, LPG, firewood and fuel (petrol and CNG) used in personal vehicles (car and motorcycle) with reference to Pakistan have been taken from Carbon Neutral Company (2014), DEFRA (2012) and Intergovernmental Panel on Climate Change's (IPCC's) (2006).

Regression analysis has also been carried out to show the impact of household income (standard of living) and other socioeconomic and demographic factors as independent variables on carbon dioxide emissions estimated as dependent variables (Buchs & Schnepf, 2013; Kavi & Viswanathan, 2013). To this end, the following model has been estimated:

$$\ln CO^{hh}{}_{2e} = \beta_0 + \beta_1 Y_h + \beta_2 Y_h{}^2 + \beta_3 HS + \beta_4 HS * Y_h + \beta_5 ED + \beta_6 ED * Y_h + \mu_i$$

Where $CO^{hh}{}_{2e}$ are the household CO_2 emissions in kg per month used as an indicator of the environmental degradation. Y_h is the household income in rupees per month used as an indicator for household standard of living and its expected sign is positive. $Y^2{}_h$ is the squared term of the household income to estimate the existence of the environmental Kuznets curve (EKC) at household level. The expected sign of the squared term of the household income is negative. HS is the household size in number and its expected sign is positive. $HS * Y_h$ is the interaction term of the household size with household income. The expected sign of this is positive. ED is the number of years in education of the main income provider and its expected sign is negative. $ED * Y_h$ is the interaction term of education of the main income provider with household income and the expected sign of this is negative and μ_i is the error

term. Firstly the model is estimated for overall district Mardan and then estimated for rural and urban households of district Mardan separately.

Results and Discussion

In urban areas, the average CO_2 emissions were higher from the usage of air conditioner, refrigerator and freezer because these are mainly used by urban households and also because of the energy-intensive lifestyle of the urban households (Table 1). This means that in the electrical appliances the more polluting items are air conditioner, refrigerator and freezer in urban areas of district Mardan. Similarly the usage of personal transportation also results in higher CO_2 emissions. This is due to the fact that majority of the households have personal transportation facility in their homes.

Table 1. Average household size, income, education and CO_2 emissions in Mardan district

Variable	**Unit**	**Rural**	**Urban**
Monthly household income	Rs.	46285.211	66727.300
Household size	Number	6.671	5.722
Education of the main income provider	Years	11.427	12.056
CO_2 Emissions of HH (kg) per month			
Sui Gas	kg CO_2e	0.734	0.842
Firewood	kg CO_2e	164.645	15.748
LPG	kg CO_2e	13.035	4.892
Personal Transport	kg CO_2e	535.769	68.997
Waste	kg CO_2e	17.431	8.639
Electrical Appliances			
Fans	kg CO_2e	38.689	16.726
Air Conditioner	kg CO_2e	106.951	129.016
Air Cooler	kg CO_2e	19.358	18.180
Energy Saver	kg CO_2e	6.522	7.357
Bulb	kg CO_2e	37.706	25.629
Washing Machine	kg CO_2e	2.506	1.559
Refrigerator	kg CO_2e	96.810	96.582
Freezer	kg CO_2e	64.773	123.082
Television	kg CO_2e	22.554	15.768
Iron	kg CO_2e	8.301	8.759
Vacuum Cleaner	kg CO_2e	37.297	6.993
Geyser	kg CO_2e	54.703	75.528
Electric Heater	kg CO_2e	31.648	55.158
Computer	kg CO_2e	20.975	13.203
Laptop	kg CO_2e	10.949	12.110
Microwave oven	kg CO_2e	44.757	46.000

On the other hand, in rural areas of district Mardan, the lowest CO_2 emissions are from Sui gas. The usage of personal transportation has higher average CO_2 emissions than urban areas of district Mardan. This is due to the fact that rural households are far away from different types of facilities like school, colleges, hospitals, etc. which are situated in urban areas. Therefore majority of the households have personal transportation in their homes. The firewood emissions are also higher in rural than in urban areas because of the low access of Sui gas to rural areas. The average CO_2 emissions of waste in rural area is higher than in urban area because of the large household size.

In the overall Model, the coefficient of income is positive and significant depicting that with the increase in household income, CO_2 emissions increases (Table 2). An inverted U shaped relationship exists between CO_2 emissions and income at household level. This means that as household income increases, the CO_2 emissions also increases, ultimately reaching to a turning point where the household CO_2 emissions then start to decline with a further increase in household income. So, as standard of living improves the environmental degradation increases. Household size is also positive and significant which is in line with the results of Wu, Liu, and Tang (2012) but in contrast to the findings by Li and Wang (2010). The interaction term of income and household size is negative and significant. The education coefficient is positive and significant showing that if the main income provider of a household is educated then the household emits more CO_2 emissions. This finding is in contrast to Baiocchi et al. (2010) but in accordance to Buchs and Schnepf (2013). This positive link between education and household CO_2 emissions is due to the fact that as the households are getting more educated, they move to a high social status and a more carbon intensive consumption pattern. The interaction term of income and education is negative and significant which is also in line with Grunewald, Harteisen, Lay, Minx, and Renner (2012).

Table 2. Regression results of the influencing factors of CO_2 Emissions in Mardan

Variable	**Overall**	**Urban**	**Rural**
	Coefficient	**Coefficient**	**Coefficient**
Income	0.0000177***	0.0000173**	0.0000152**
$Income^2$	-0.00000000002**	0.0000000000019	-0.000000000036***
Household size	0.1304098***	0.120808**	0.1278079***
Income*Household size	-0.00000105**	-0.00000121**	-0.00000081*
Education	0.0261412*	0.0472491**	0.000293
Income*Education	-0.000000401**	-0.000000688*	-0.0000000973
Constant	5.636288***	5.718356***	5.803158***
R^2	0.1371	0.0825	0.1822
F-Statistics	10.22	2.73	7.65
Prob. F-Statistics	0.0000	0.0146	0.0000

Note: *, **, *** 10%, 5% and 1% level of significance respectively. Dependent Variable = CO_2 Emissions (Kg).

In the urban areas, the coefficient of income is positive and significant. The coefficient of income squared term is positive and insignificant implying that the hypothesis of Environmental Kuznets curve (EKC) does not hold true in urban areas of district Mardan. This result is in line with the result of Kavi and Viswanathan (2013). The reason for this is that the urban households follow a carbon intensive lifestyle which restricts the relationship between income and CO_2 emissions to the upward sloping segment of the EKC. The coefficient of household size is also positive and significant. The interaction term of income and household size is also negative and significant in urban areas showing that higher income and larger household size contribute to less CO_2 emissions. Because as the household become wealthier they prefer a clean environment. The coefficient of education in urban areas is positive and significant. Similarly, the coefficient of interaction term of income and education is negative and significant in urban areas.

In rural areas, the coefficient of income is positive and significant further implying that there exists an inverted U shaped relationship between income and CO_2 emissions and so, the EKC exists in rural areas of district Mardan. The coefficient of household size in rural areas is also

positive and significant. The interaction term of income and household size has a negative and significant coefficient. The coefficient of education is positive and insignificant in rural areas. The coefficient of the interaction term of income and education is negative and insignificant. Both education and the interaction term of income and education are insignificant showing that the education level of rural households is not high so the resulting social status is also not high which is associated to a high level of education. As the social status of the rural households is not high so their consumption pattern is also less energy intensive, therefore, contributing to less CO_2 emissions.

Conclusion

Based on findings it is concluded that environmental degradation increases with improvement in the standard of living. Both household size and education positively and significantly affect the CO_2 emissions. The EKC is valid in rural areas but not in urban areas. The influencing factors CO_2 emissions at household level were mainly income, household size and education in district Mardan. To have sustainable standard of living, households should be aware of the environmental impacts of their consumption choices and activities. This can be done through media and public campaigns. Consumers should be given incentive for choosing energy saving goods and services. These incentives can be given in the form of tax reductions for rewarding them to save the energy.

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BIODIVERSITY POTENTIAL IN CAMPING DESTINATIONS CORBU AND VADU FROM DANUBE DELTA BIOSPHERE RERSERVE

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Abstract

Corbu and Vadu, two villages from Constanţa County, Romania are recently camping destinations for those tourists seeking natural beauty and the concept of virgin beaches on the Black Sea.

In July 2013 and August 2014, we observed, studied and collected some biological material from these sites situated in the southern part of Danube Delta Biosphere Reserve. Considering the biodiversity potential, we have compiled for the Corbu-Vadu are an inventory of over 23 vegetal families representative for local vascular flora and eight insect orders (Coleoptera, Heteroptera, Homoptera, Hymenoptera, Lepidoptera, Mantodea, Neuroptera, Orthoptera), with special insight on insect-host associations like *Eurydema spectabilis – Crambe maritime*, *Carpocoris pudicus – Eryngium maritimum*, *Rhagonycha fulva – Daucus carota*. From mollusks species, we found the rarer razor clam *Solen marginatus* along the Vadu beach.

As regarding the avifauna, in the summer of 2013, we recorded in the Vadu area 92 species belonging to 13 orders and 35 families of birds. The most abundant in species composition was the order of Charadriiformes.

The impacts of human activities on the protected landscapes and the hygienic conditions of the camping trips in these seaside areas are also discussed.

Keywords: *Danube Delta Biosphere Reserve, biodiversity, anthropogenic impact, beach*

Introduction

The Corbu and Vadu areas, as parts of Black Sea shore, found in the south limit of Danube Delta Biosphere Reserve and in the central Dobroudja region, are home for protected species of fish, amphibians, reptiles and birds but also for insects and vascular flora (Dinu et al., 2009; Făgăraş and Jianu, 2007).

Făgăraş (2008) recorded for Corbu-Vadu seacoast, an area with a surface about 50 hectare, 24 plant associations and 173 number of plant taxa. Unfortunately, in Dobroudja, the natural vegetation has been deeply affected due to the long-termed anthropogenic influence, like tourism and agricultural activities (Nonev, 2008).

In Dobroudja and Danube Delta, there are the highest concentrations of birds in Romania and even in Europe, due to preferred migration corridors and favorable areas for feeding, resting and breeding. The localities from Corbu commune (Corbu, Luminiţa and Vadu) there are just on two from the three main migration routes in Dobroudja, namely Sarmatic route – crossing the Danube Delta and following the seashore, and Sarmatic Maritim, branching directly across the Black Sea to the north. These corridors are used by both spring and autumn migrations (Dinu et al., 2009; Ion et al., 2009). At the same time, the aestival season represents a disturbing factor for the nesting birds in the Romanian coastal area (Bănică, 2008).

It is well known that tourism could have a negative effect on the natural resources in camping destinations from DDBR (http://www.danubeparks.org/). The dunes habitats can be destroyed through the expansion of the touristic activities (Făgăraş et al., 2008).

Regarding the Vadu area, a recent report (TRANSDANUBE, 2014) drew attention to the fact that currently there is an uncontrollably camping activity, that threatens the conservation status for the coastal habitats.

Material and method

Our observations were made in field trips carried out in: 22.07.2013-29.07.2013 (Corbu area – 44°22'44.01"N, 28°42'43.51"E; 44°22'46.64"N, 28°42'43.67"E) and 25.07.2013-30.07.2013, 15.08.2014-17.08.2014, 22.08.2014-27.08.2014 (Vadu area – 44°25'41.70"N, 28°45'53.99"E; 44°25'46.64"N, 28°46'1.41"E; 44°25'47.53"N, 28°46'0.20"E; 44°25'48.73"N, 28°46'4.75"E; 44°25'58.17"N, 28°46'8.98"E; 44°26'38.19"N, 028°44'45.01"E; 44°28'07.14N", 028°45'01.77E").

Biological material, like insects associated with vascular flora, was observed *in situ* and collected from the following categories of habitats: sandy beaches, sand dunes with psammophile vegetation, ruderal places, coastal wetlands and ponds, bushes area along the marine shore line.

Direct observation was the most used method in this type of qualitative research, in order to focus on normal behavior and adaptations of the living organisms in their natural environment (Dobrin et al., 2013). Like any qualitative data analysis, our survey was non-statistical, its methodological approach being guided by the concrete material at hand; sample selection was based on smaller cases, compared to a quantitative research (http://atlasti.com/quantitative-vs-qualitative-research/). Numbers were used to describe a sample and to generate meaning from the data (Sandelowski, 2001). This technique, applied for collecting data about various features and processes, included photographing and videotaping; scientific and vernacular names of the recognized biological specimens were mentioned in a field notebook.

Some steps in sorting and classification of species were conducted in the field, other in laboratory.

The nomenclature for the vascular flora identified in the studied areas is in accordance with the Romanian Flora and Flora Europaea (Ciocârlan, 2000; http://rbg-web2.rbge.org.uk/cgi-bin/nph-readbtree.pl/). Plant specimens were harvested by hand pulling, some being dried and pressed in a herbarium or preserved in alcohol, in order to identify them in the laboratory work.

Insect species, captured with an entomological net, have been identified according to Leraut (2012); some of them were whole preserved in alcohol, prior to study them in UASVM Bucharest laboratories.

For the ornithological study, were used binoculars and spotting field telescopes, working out visual transects to identify birds. The book-guides used are Bruun et al. (1999) and Svensson et al. (2010). In July 2013, the observations were made twice a day, around 7 a.m. and 5 p.m., in various points from Vadu area, such as: the beach, accumulations basins, swampy habitats, village and the area around the neighboring forest.

Also, some bird species included in this research have been reported since the summer of 2007 (Mihai, unpublished data), in the camps organized by the Romanian Ornithological Society (ROS), an organization that aims to study and protect the wild birds. It is already know that the objectives of these camps include monitoring of the bird migration through ornithological observations and ringing activities, and at the same time popularization of ornithology and environmental protection (http://www.sor.ro).

Results and discussion

Our survey presents findings descriptive in nature, involving recording presence of flora and fauna species and the relationship between these components of biological diversity. Similar

approaches were used worldwide by researchers like Bieler and Mikkelsen (2004), Golodets et al. (2013) or Grant and Shepard (1985).

Vascular flora. In the Corbu area, the following plant taxa have been identified in our survey conducted in July 2013: Asteraceae (*Artemisia herba-alba, Centaurea* sp., *Cichorium intybus*, *Cirsium vulgare*, *Xeranthemum annuum*); Apiaceae (*Eryngium maritimum*); Caprifoliaceae (*Scabiosa argentea*); Convulvaceae (*Cuscuta campestris*); Cyperaceae (*Scirpoides holoschoenus*); Elaeagnaceae (*Elaeagnus angustifolia*); Euphorbiaceae (*Euphorbia nicaeensis* ssp. *dobrogensis*); Fabaceae (*Astragalus varius*, *Melilotus albus*); Gramineae (*Calamagrostis epigejos, Leymus racemosus* ssp. *sabulosus, Phragmites* sp.); Labiatae (*Teucrium polium* ssp. *capitatum*); Linaeceae (*Linum austriacum*); Lythraceae (*Lithrum salicaria*); Rosaceae (*Potentilla anserina, Crataegus monogyna*).

In the Vadu area, the following plant families and species have been identified in August 2014: Asteraceae (*Achillea millefolium*, *Artemisia arenaria*, *Centaurea arenaria*., *Onopordum acanthium*, *Scolymus hispanicus*, *Senecio grandidentatus*, *Senecio jacobaea*, *Xanthium italicum*, *Xeranthemum annuum*); Amaranthaceae (*Salicornia herbacea*, *Salsola kali*, *Suaeda maritima*); Apiaceae (*Eryngium maritimum*); Apocynaceae (*Cynanchum acutum*); Brassicaceae (*Crambe maritima*); Caprifoliaceae (*Scabiosa argentea*); Caryophyllaceae (*Dianthus bessarabicus*); Elaeagnaceae (*Elaeagnus angustifolia*, *Hippophae rhamnoides*); Fabaceae (*Astragalus varius*); Gramineae (*Leymus racemosus* ssp. *sabulosus*, *Phragmites* sp.); Juncaceae (*Juncus* sp.); Labiatae (*Ajuga genevensis*, *Teucrium polium* ssp. *capitatum*); Linaceae (*Linum perene*); Lythraceae (*Lithrum salicaria*, *Trapa natans*); Plantaginaceae (*Linaria genistifolia*); Plumbaginaceae (*Limonium ramosissimum*); Polygonaceae (*Polygonum arenarium*); Potamogetonaceae (*Potamogeton crispus*, *Zannichellia palustris*); Rosaceae (*Crataegus monogyna*); Scrophulariaceae (*Verbascum nigrum*); Typhaceae (*Typha* sp.).

As ROS/BirdLife showed (http://www.rspb.org.uk/Images/danubedelta_tcm9-227929.pdf), *Crambe maritima*, *Leymus racemosus* ssp. *sabulosus* and *Eryngium maritimum*, parts of the herbaceous vegetation of the coastal dunes, are globally threatened plant species, being affected by the inadequately controlled economic development and tourism.

Within the coastal wetland of Corbu-Vadu area *Lythro-Calamagrostetum epigeix* represents a main mesophilous plant association, while *Scirpo-Phragmitetum* is among the most important hygrophilous plant communities, as Făgăraş (2008) also mentioned in a previous research.

Insect records. The insect taxons from Corbu, sampled in particular in the reed of the neighboring beach are: Coleoptera (Cantharidae: *Rhagonycha fulva*, Curculionidae: *Apion apricans*, Tenebrionidae: *Blaps mucronata*, Coccinelidae: *Coccinella septempunctata*, Meloidae: *Mylabris floralis*, Melolonthidae: *Polyphylla fullo*); Heteroptera (Pentastomidae: *Ancyrosoma albolineatum*, *Aelia acuminata*, *Dolycoris baccarum*, Scutelleridae: *Eurygaster* spp., Lygaeidae: *Lygaeus equestris*, Nepidae: *Ranatra linearis*, Rhopalidae: *Rhopalus* spp.); Homoptera (Cicadellidae: *Asiraca clavicornis*); Orthoptera (Tettigonidae: *Decticus verrucivorus*, Acrididae: *Dociostaurus maroccanus*); Neuroptera (Chrysopidae: *Chrysopa perla*).

From the dune vegetation of Vadu area, we sampled and identified so far the following insect taxons: Coleoptera (Chrysomelidae: *Cassida nebulosa*); Heteroptera (Pentatomidae: *Dolycoris baccarum*, *Eurydema spectabilis*); Homoptera (Cicadellidae: *Macrosteles sexnotatus*); Hymenoptera (Vespidae: *Vespa velutina*); Lepidoptera (Lymantriidae: *Leucoma salicis*, *Penthophera morio*); Mantodea (Mantidae: *Mantis religiosa*); Orthoptera (Acrididae: *Calliptamus italicus*; Gryllidae: *Gryllus campestris*; Tettigonidae: *Decticus verrucivorus*, *Tettigonia viridissima*).

Mollusk fauna. On both beaches, we noticed species of Gastropoda (*Cyclope neritea*, *Hinia reticulata*, *Rapana venosa*) and Bivalvia (*Anadara* spp., *Cerastoderma edule*, *Chamelea*

gallina, Donax trunculus, Mya arenaria, Mytilus galloprovincialis, Ostrea taurica, Spisula spp., *Tellina tenuis*). The empty shells of european razor clam, *Solen marginatus*, characteristic for slightly muddy fine sands, was found in Vadu beach and has been reported so far only in few sites from the Romanian coastline (NIMIRD Report, 2011).

Avifaunistic diversity in Vadu area. In recent years, this area has been constantly visited, in individual filed trips or as active member (Mihai, unpublished data, 2007-2013) in the ornithological camps organized by ROS. At the end of July 2013, were recorded 92 species of birds belonging to 35 families, as follows: Podicipedidae (*Podiceps cristatus, P. nigricollis, Tachybaptus ruficollis*); Pelecanidae (*Pelecanus crispus, P. onocrotalus,*); Phalacrocoracidae (*Phalacrocorax carbo, P. pygmeus*); Ardeidae (*Ardea cinerea, A. purpurea, Ardeola ralloides, Casmerodius albus, Egretta garzetta, Ixobrychus minutus, Nycticorax nycticorax*); Threskiornithidae (*Plegadis falcinellus, P. leucorodia*); Ciconiidae (*Ciconia ciconia*); Anatidae (*Cygnus olor, Anser anser, Anas plathyrynchos, A. strepera, A. penelope, A. crecca, A. clypeata, Tadorna tadorna, T. feruginea, Aythya ferina, A. nyroca*); Accipitridae (*Buteo buteo, B. rufinus, Circus aeroginosus*); Falconidae (*Falco subbuteo, F. tinnunculus*); Rallidae (*Fulica atra, Gallinula chloropus*); Haematopodidae (*Haematopus ostralegus*); Charadriidae (*Charadrius alexandrinus, C. dubius, C. hiaticula, Vanellus vanellus*); Scolopacidae (*Actitis hypoleucos, Calidris alba, C. ferruginea, C. minuta, C. temminckii, Gallinago gallinag, Limosa limosa, Limicola falcinellus, Numenius arquata, Philomachus pugnax, Tringa stagnatilis, T. totanus*); Recurvirostridae (*Himantopus himantopus, Recurvirostra avosetta*); Glareolidae (*Glareola pratincola*); Laridae (*Larus argentatus, L. melanocephalus, L. ridibundus*); Sternidae (*Chlidonias hybridus, C. leucopterus, C. niger, Sterna albifrons, S. hirundo, S. sandvicensis*); Columbidae (*Streptopelia decaocto*); Cuculidae (*Cuculus canorus*); Strigidae (*Athene noctua*)*;* Meropidae (*Merops apiaster*); Coracidae (*Coracias garrulus*); Picidae (*Jynx torquilla*); Alaudide (*Galerida cristata*); *Hirundinidae* (*Hirundo rustica*); Motacillidae *(Motacilla alba, Motacilla flava);* Laniidae (*Lanius collurio*); Sturnidae (*Sturnus vulgaris*); Corvidae (*Corvus corone cornix, C. monedula, Pica pica*); Sylviidae (*Acrocephalus arundinaceus, A. schoenobaenus, Locustella luscinoides, Phylloscopus collybita, P. sibilatrix, Sylvia curruca*); Muscicapidae (*Muscicapa striata*); Turdidae (*Erithacus rubecula, Oenanthe oenanthe*); Timaliidae (*Panurus biarmicus*); Passeridae (*Passer domesticus, P. montanus*); Emberizidae (*Emberiza schoenilus*).

It should be noted that, over the years, in Vadu area we had the opportuniy to observe, in different seasons, extremely rare species of birds for Romanian fauna. In order to highlight the importance of this area for global avifauna, some birds worth mentioning are terek sandpiper (*Xenus cinereus*), observed in 16.08.2007 and also yellow-browed warbler (*Phylloscopus inornatus*) which could be seen in 29.09.2013.

Besides the Scolopacidae, other abundant in species composition were Anatidae and Ardeidae bird families. Our results can be compared with those of Dorosencu et al. (2004), who observed 177 species of birds in Vadu area, between 18.08.2003 and 01.09.2003. Like in this previous study, we found that the most well represented was the order of Charadriiformes.

Ecological relationships in the study area. In both observed sites, the associations of bushes with *Eleagnus angustifolia* and *Hippophae rhamnoides*, which grow along the sand banks, are important feeding and resting place during the migration of the passerines species (Dorosencu et al., 2004).

The sea buckthorn (*Hippophae rhamnoides*), whose distribution is due to frugivorous birds and vegetative spreading, it is now considered a plant species for dunes formations and stabilization (Strat, 2013).

A typical plant adapted to sand and salt soil, the sea holly (*Eryngium maritimum*), represents an important shelter for insect populations, the main source of food for insectivorous (*Sylvia curruca, Phylloscopus collybita, Phylloscopus sibilatrix, Muscicapa striata, Oenanthe*

oenanthe, Erithacus rubecula) and omnivorous (*Passer domesticus, Sturnus vulgaris, Cuculus canorus)* birds (Ion et al., 2009).
As regarding insect-plant associations, we encountered the trophic relationships between the shield bug (*Eurydema spectabilis*) and the bushes of sea kale (*Crambe maritima*) (Figure 1 a, b) and also between *Carpocoris pudicus* and the sea holly (*Eryngium maritimum*) (Figure 1 c). Both pentatomid species populate the terrestrial littoral biotopes like beach strips with herbaceous vegetation (Skolka, 2008). Also, in the neighboring wetlands of the Corbu beach, the common red soldier beetle (*Rhagonycha fulva*) is a pollinator visiting the inflorescence of the wild carrot (*Daucus carota*) (Figure 1 d).

Figure 1. Insect-plant associations in the study area: a, b: *Eurydema spectabilis* on sea kale (Vadu), c. *Carpocoris pudicus* on sea holly (Vadu), d. *Rhagonycha fulva* on wild carrot (Corbu).

Anthropogenic impact. The human impact is signaled in particular by the garbage thrown by tourists near their tents, cars or trailers parked on the beach. Travelling by cars, motorbikes or boats near the birds' colonies, waste and camping are among the pressures involved in Vadu-Corbu area (TRANSDANUBE, 2014).
As informative and awareness panels installed on Corbu and Vadu beaches recall, camping is permitted only in authorized campsites of the DDBR, properly equipped according to sanitary requirements (www.ddbra.ro/). Also, abandoning waste and plastic packaging, paper, metal or glass is not allowed and is financially penalized. However, every year, the behavior of some tourists proves otherwise (Figure 2). In addition, the disgusting exposed feces, surrounded by used toiled paper, randomly thrown in vegetation, besides the deterioration of the beauty and the balance of natural landscape, could also have negative implications upon the human health.
In terms of helping the affected ecosystems, a good thing seems to be the involvement of volunteers in greening activities. It is a tradition that the Romanian Ornithological Society organizes in the Danube Delta and the sea shore area Vadu-Chituc each year the National

Ornithological Camp, whose members conducting a campaign of collecting the trash on the beach (Figure 3).
Unfortunately, measures to protect the biodiversity of this area are almost nonexistent. Access to the Corbu-Vadu area was not restricted in the last years, leading to abuses made by poachers and people who vandalize plants and any creatures they meet.

Figure 2. Human waste persists in the protected areas of DDBR, despite warnings.

Figure 3. Waste collection campaign in Vadu area, organized by Romanian Ornithological Society.

On the other hand, as there is no management plan in this area, travelers departing leave behind mountains of trash, which mostly affect wildlife fauna or birds that can swallow non-digestible pieces of rubbish, like sharp plastic or glass shards. Moreover, there are Romanian and foreign companies interested in developing residential and leisure complex on the Vadu beach. The Romanian media and the environmental NGOs make desperate efforts to bring to light the abuses from political and administrative environment. First, these constructions violate the administrative laws of the protected areas and perhaps most important is that such buildings in the protected area of DDBR will have negative and irreversible effects upon the important natural heritage worldwide.

Conclusion

Without being exhaustive, the present study still brings a solid contribution to the knowledge of the recent biodiversity in Corbu-Vadu protected zones from the Danube Delta Biosphere Reserve. As other papers and reports pointed out, these sites of the Romanian seacoast offer excellent opportunities both for responsible camping in nature and specialists research (botanists, zoologists, ecologists).
The biological inventory compiled during the summers of 2013 and 2014 included vascular flora, invertebrates (insects, mollusks inhabiting the sandy beaches) and birds.

In terms of the numbers of species, best represented were the following taxons: Asteraceae, Heteroptera and Charadriiformes. Regarding the clams, in Vadu seashore we found empty shells of *Solen marginatus*, a rare presence on Romanian coastline.

Our qualitative survey highlighted also few trophic adaptations of some living organisms in Corbu-Vadu area, like pentatomid bugs associated to their wild plant hosts. Among ecological relationships established in the studied zones, we found that sea buckthorn bushes are important feeding and resting place for the passerines species, while the sea holly represents a good shelter for insect populations, the main source of food for insectivorous and omnivorous birds.

Unfortunately, the anthropogenic impact (feces and toilet paper thrown in vegetation, garbage left by tourists near their tents or cars parked on the beach) is destroying the human health and natural ecosystems. Thus, typical plants of the coastal dunes, *Eryngium maritimum* and *Crambe maritima* seem to be affected by the inadequately controlled economic development and tourism.

Luckily, the members of the National Ornithological Camp organized each year by the Romanian Ornithological Society, lead a campaign of collecting the human waste on the Vadu beach.

Considering the species richness in the area, in near future it should be considered special management measures, in order to protect the environment and limiting the access of the tourists.

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THE RECYCLING AND EVALUATION OF LF SLAG FOR SOIL ACIDITY NEUTRALIZATION

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Abstract

The aim of the current research was to investigate the properties of some new materials in order to improve the soil acidity and to evaluate to effect of slag from steel refinery on chromic luvisol physico-chemical properties.

Yearly, in Romania are produced from 0.18 up to 0.2 million tonnes of ladle slag that is classified as a dangerous waste due to increased alkalinity. Ladle slag (LF slag) results from steel secondary refinery and cannot be recycled in the process because the iron content is too small. In the same time, ladle slag contains high percent of calcium oxide and other calcium compounds.

Having in view those above mentioned, was developed an experiment at the experimental field from Moara Domneasca using ladle slag as amendment that induce a correction of acidic reaction of soil.

The experiment was designed with five experimental variants with three replicates for each variant: V_1 (control), V_2(1 t/ha), V_3(2 t/ha), V_4(3 t/ha) and V_5(5 t/ha). The biological material used in this experiment was maize in 2013 and wheat in 2014.

The results indicated that slag can be used successfully to correct soil acidity, to improve the main physico-chemical properties of soil and the increase of soil fertility.

Slag from the steel industry brings increases of calcium and micronutrients levels in the soil.

There was an increase in the reaction of chromic luvisol with increasing rate from 5.7 to 6.72 at 20-40 cm depth. The highest yield of maize, 7.97 t ha^{-1}, was obtained in V_4 (5 t/ha) and biomass production was higher in treated variants compared to the control variant. Largest wheat yield increases were achieved as a consequence of the rate of 5 t/ha with nitrogen mineral fertilizers.

Keywords*: recycling, LF slag, soil, acidity, wheat, maize*

Introduction

Utilization of industrial waste materials in order to improve problematic soils is cost efficient and environmental friendly method, as well; it helps in reducing disposal problems caused by the various industrial wastes. The surface of the soil suffers an acidification process especially under no liming conditions, soil pH values less than 5.5 ensuring conditions for strong solubilization of pollutants and their translocation into plants. In Romania more 2 million ha are soils with moderately acid and highly acid reaction (Fig. 1).

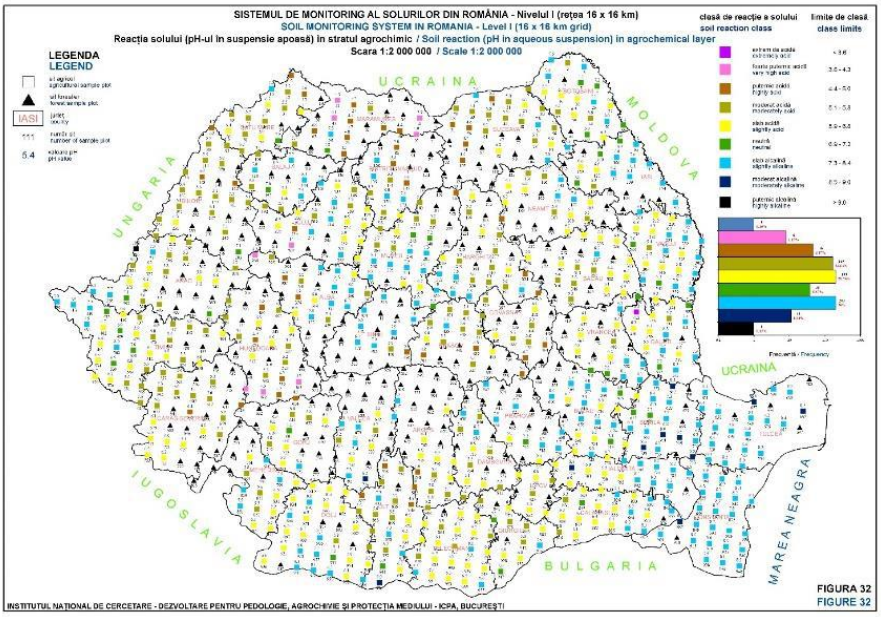

Figure 1. Soil reaction in agrochemical layer from Romania

Yearly, about 45 million tons of LF slag are generated in Europe (www.euroslag.com).
The steel industry produces not only metal, but also by-products which have been successfully used for construction and/or agriculture purposes.
The awareness of environmental considerations and more recently the concept of sustainable development as well as the need for recycling of by-products for economic and environmental reasons has led to rapid development of slag utilization.
The use of fertilizers and liming materials produced from blast furnace and steel slag has a long tradition. For example Thomas phosphate had been used for more than hundred years.
Silicate lime fertilizers (blast furnace lime and converter lime) are approved fertilizers in Germany for more than 60 and 30 years, respectively. They contain elements with useful properties for plant nutrition and soil quality.
The solubility of silicate from slag is often higher than from many other silicate containing soil improvers or rock powders. Blast furnace lime has a mild effect on soil pH. Therefore, it is recommended especially for use on humus, sandy and peat soils. In addition, the high silicate and magnesium content which is readily available to plants promotes yields and plant qualities.
The basicity of the calcium and magnesium compounds in the slag improves soil pH. Both elements also serve as plant nutrients and stabilisers for soil aggregates. Magnesium in slags especially in blast furnace slag has a better solubility than that of magnesium carbonate in limestone and dolomite. Silicate has beneficial effects on plant health, phosphate availability and soil structure. The content of trace elements like manganese, copper, zinc, boron or cobalt satisfies both plant and animal demands (Rex M., 2010).
Mohammadi T. concluded that these materials used on the acid soil increased soil pH and DTPA extractable P more than paper mill sludge (PMS) and increased Mn proportional to the slag application rate, while PMS decreased it.
Aim of this study was testing impact of LF slag application on soil properties and yields of maize and wheat in rotation.

Materials and methods

The field experiment

The stationary field experimental was started in autumn 2012 in the experimental field of the Faculty of Agriculture from Moara Domneasca on chromic luvisol (Fig. 2 and 3). Four rates of LF slag (1, 2, 3 and 5 t^{-1}) and control (without LF slag) were applied. The experimental was performed by randomized block in three replicated (basic plot has 30 m^2). Maize (LG 30.489 hybrid) was grown in the first year (2013) while winter wheat (*Glosa* variety) was grown in the second year of the experiment (2014). The experimental plot was fertilized as follows (kg/ha^{-1}): without mineral fertilized at maize and $100N+0P_2O_5+0K_2O$ (wheat).

Figures 2,3. Experimental field from Moara Domnească, Ilfov County (2013)

Soil characteristics before starting of the experiment

The chromic luvisol is characterized by a clay loam texture, organic matter content of 2.4% and a low acid pH with values of 5.0-5.4 (Mihalache et al., 2013).

Sampling, chemical and statistical analysis

Samples of LF slag were analyzed by Optic Spectometry with plasma inductively coupled ICP-OES, for iron, magnesium, aluminium, silica etc.

In order to show the amendment effect on the main chemical properties of chromic luvisol, soil samples on two depths were taken (0-20 cm and 20-40 cm) from all basic plot. The following soil measurements were performed: the soil reaction (pH) was carried out through potentiometric method, in an aqueous suspension, 1:2.5 (w/v). For determining the reaction of the soil it was used WTW 750 pH-meter; organic carbon was determined using the Walkley-Black-Gogoasa method; humus content was calculated (C_{org}x1.724); phosphorus content was evaluated by spectrophotometric molybdenum blue method, using ammonium acetate-lactate (AL) as extractive solution, at pH 3.7 (Egner-Riehm-Domingo method); total form of nitrogen was determined using Kjeldahl method, meanwhile total phosphorus was assessed spectrophotometrically (as molybdenum blue) and potassium using flame photometry method. Soil samples were collected in the 0-20 cm and 20-40 cm depth for all experimental variants to follow influence on soil reaction and the wheat yield. The obtained data were statistically processed by analyses of variance.

Results and discussions

The material used as amendment has a high content of Ca 43.50% and reaction is strongly alkaline (pH=11.08). In addition, to the high content of calcium, the material has a high content of magnesium and iron, as well. The chemical composition of LF Slag used in experimental field is presented as it follows: Fe 10.55%; Mg 4.10%; Al 2.27%; Si 8.70% and Na 0.034%. The heavy metals found content is less than maximum allowable limits.

Amendment application on the chromic luvisol from Moara Domneasca produced an increase of soil reaction at 0-20 cm depth from 5.51 in control variant up to 6.01 in variant with 5 t/ha of ash applied (table 1). A significant increase of soil reaction was recorded since at the first year of slag application at 20-40 cm depth the pH rised from 5.55 in control variant to 6.35 in variant with 5 t/ha of LF slag applied.

Table 1. The effects of LF slag upon main chemical properties of chromic luvisol (2013)

LF slag t/ha^{-1}	Soil depth 0-20 cm					Soil depth 20-40 cm				
	pH $_{H2O}$	Percent		mg kg^{-1}		pH $_{H2O}$	Percent		mg kg^{-1}	
		C org	N	P	K		C org	N	P	K
0	5.51	1.21	0.147	74	230	5.55	1.23	0.158	87	235
1	5.68	1.35	0.157	106	225	5.77	1.31	0.153	108	270
2	5.80	1.28	0.153	97	298	5.92	1.23	0.155	105	287
3	5.78	1.25	0.151	85	299	5.95	1.21	0.157	87	326
5	6.01	1.32	0.157	78	311	6.35	1.25	0.132	89	317
	LSD values					LSD values				
5%	0.016	0.019	0.0011	1.68	1.64	0.015	0.023	0.003	2.062	1.898
1%	0.024	0.028	0.0016	2.45	2.40	0.023	0.034	0.004	3.001	2.762
0.1%	0.036	0.042	0.0024	3.68	3.60	0.035	0.051	0.006	4.509	4.150

At 20-40 cm depth the phosphorus content increased from 87 mg kg^{-1} to 108 mg kg^{-1}in the case of application of 1 t/ha of amendment. Significant increases of soil phosphorus content were recorded in all experimental variants in comparison with control variant (no limestone amendment application).

Maize yield was positively influenced by the application of different rates of LF slag, in the control variant being 72.15 q/ha; in variant V_5 (5 t/ha) the yield increased to 79.66 q/ha. Yield growth was 4.13 q/ha to V_2 (1 t/ha), 2.83 q/ha to V_3, 2.1 q/ha to V_4 and 1.50 q/ha to V_5 (5 t/ha).

Table 2
Impact of LF slag application on maize (the 2013 crop year) and wheat (2013/2014 crop year)

LF slag t/ha^{-1}	Maize (2013) yield t/ha^{-1}	Wheat (LF slag +0 N and LF slag +100 kg N ha^{-1})					
		Winter wheat yield t/ha^{-1}		Ears number per square meter		Soil pH (H_2O)	
		0 N	100 N	0 N	100 N	0 N	100 N
0	7.22	4.6	6.8	482	615	5.88	5.69
1	7.63	4.9	6.9	509	643	6.04	5.84
2	7.78	5.3	7.2	522	640	6.13	5.73
3	7.85	6.1	7.3	582	650	6.19	5.91
5	7.97	6.3	7.9	600	664	6.89	6.22
LSD Values							
5%	0.31	0.32	0.30	3.36	7.69	0.06	0.13
1%	0.45	0.46	0.45	4.90	11.19	0.09	0.19
0.1%	0.68	0.70	0.67	7.36	16.89	0.14	0.29

In 2014, the amendment application improved the chemical properties of chromic luvisol by increasing soil reaction with slag application rate (Table 2).

Figures 4. The variation of soil reaction on the depth 0-20 cm (2014)

High yields were obtained due to the residual effect of amendment and application of 100 kg of nitrogen. The highest yield was obtained by applying a LF slag rate of 5 t/ha. Our opinion is that applying LF slag rates of 3 t/ha are recorded yield increases that are significant under soil and climatic conditions of Moara Domneasca (Fig. 4).

Direct application of various rates of slag caused, depending on the rate applied, better wheat crop development, a greater number of ears and a significant increase of wheat yield in 2014 from 4.6 t ha $^{-1}$ at control variant to 6.3 t ha $^{-1}$ in the variant with 5 t/ha LF slag. The number of wheat ears was between 482-600 ears/m^2.

Research conducted with various plants showed an increase of production, due to better use of plant nutrients by changing the soil reaction.

The highest yield in climatic conditions of the year 2014, were recorded by applying slag and fertilization with 100 kg nitrogen, respectively by applying the LF slag rate of 2 t/ha the yield was 7.3 t ha $^{-1}$ and 7.9 t ha $^{-1}$ for LF slag rate of 5 t/ha. To obtain high yields are recommended amendment and mineral fertilizers application.

Conclusions

These wastes from secondary refinery of the steel can be used with good results in agriculture and with positive influences on the maize and wheat crops.

LF slag resulted from the refinery of the steel may be used as amendment for correction of the acidic soil reaction.

Slag from the steel industry brings increases of calcium and micronutrients levels in the soil.

There was an increase in the reaction of chromic luvisol with increasing rate from 5.7 to 6.72 at 20-40 cm depth.

The harvest increase per tonne of LF slag was 0.4 t ha^{-1} at a rate of 1 t/ha and 0.15 t ha^{-1} at a rate of 5 t/ha.

The highest yield of maize, 7.97 t ha^{-1}, was obtained in V_4 (5 t/ha) and biomass production was higher in treated variants compared to the control variant.

Yield increases registered on chromic luvisol under conditions of 2014, on the wheat crop, was significant due to better recovery of nutrients. Largest yield increases were achieved as a consequence of the rate of 5 t/ha with nitrogen mineral fertilizers.

The presented research have revealed that there is a residual effect of slag reflected by soil reaction and increased yields at rates above 2 t/ha.

The research that was carried out allowed us to recommend applying slag to 3-4 years this depending on the chemical composition of the slag and heavy metals content, in order to avoid environmental issues.

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Professional paper
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GRANULOMETRY FEATURES IN MOUNTAIN SOILS

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Abstract

Grain size distribution is an important physical characteristic of the soil and one of the characteristics of its dispersion. We studied the grain size distribution of mountain soils in subalpine zone in the Middle Urals. Results show grain size distribution of the soil varies from medium loamy to clay, depending on the location of the formation of soils and dominant vegetation type. In the soils under subalpine meadows marked accumulation of large and medium content of sand in the profile, which indicates that the prevalence of physical weathering and sand particles can bring to the surrounding areas as a result of denudation. Soils have a stable same structure particle size distribution with predominance of the average content of coarse dust in the structure. Statistical distribution analysis is determined for the content of sand, silt and clay fraction. The average content of clay particles in the soil under the crooked and subalpine meadows is slightly more than 15%, and in the soils of park woodlands – 30% with a maximum variation in space. Thus, the distribution of size fractions in the soil profile and their relationship to each other are diagnostic indicators of elementary soil-forming processes in the burozems, litozems, organo-accumulative soils of the subalpine zone, which are expressed in the title of the classification of soils.

Keywords*: mountain soils, subalpine meadows, elementary soil particles, clay, grain size distribution*

Introduction

Grain size distribution is a basic soil property as so as it defines all the major soil processes. Grain size distribution (GSD) expressed in terms of content of the grain size fractions is an important physical characteristic of the soil and one of the characteristics of its dispensability. The soil GSD also provides insight into the genesis, evolution and use of soils (Tatarintsev, 1998. Tatarintsev et al., 2008. 2012). Soil physical properties depend not so much on the content of physical clay (FC) but on the ratio of the grain size fractions that determine the characteristics of structure and function at higher levels of organization of the soil. The ratio of size fraction in grain size distribution, particularly sand, coarse dust, and the average amount of fine dust and sludge, N.A. Kaczynski (1958) refered to as a soil type, V.P. Panvilov (1973) and V.L. Tatarincev (1998) as structure, I.V. Mikheeva (2001, 2005, 2012) as formula, and E.V. Shein (Theories and techniques of soil physics, 2007) as a texture of grain size distribution. By the distribution of particle content by size, we can characterize the studied object detailed enough and compare different soil sites.

The purpose of the research is to determine the particle size characteristics of mountain soils in the subalpine belt in the Middle Urals.

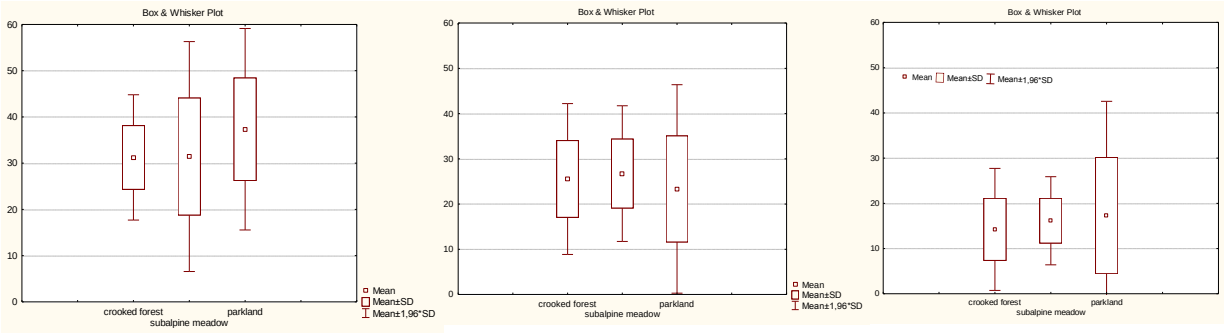
Materials and methods

We carried out the investigations in 2012-2014 on the territory of "Basegi" State National Reserve. Soil cross sections were made on the mountain of North Basegi (height 951.9 m asl) in the subalpine belt (crooked forest, subalpine meadows, parkland at an altitude of 920-557 m asl on the western macroslope of the Northern Urals. A total of 36 cross sections were made in a subgoltsy belt (8 cross sections in the crooked forest sub-belt, 18 in subalpine

meadows, 10 in parkland). Russian profile-substantive soil classification of year 2004 was used to describe and identify the names of the soils. We measured soil grain size distribution with pipette method, version of N.A. Kaczynski (with pyrophosphate method of soil preparation to the analysis). Statistical analysis of study data was performed in the "Data Analysis" software in Microsoft Excel and the STATISTICA 5.0 software.
The investigated mountain soils belong to the stem of postlitogenic soil forming. Based on morphogenetic characteristics of soils, the following formations were identified: lithosoils (layer thickness less than 30 cm), structural and metamorphic (picked horizon BM), organic-accumulative (median horizon is not pronounced as an independent genetic formation).

Results and discussion

Soil grain size distribution in the subalpine belt is lightweight medium loamy and light loamy in humus horizons and becomes heavier, heavy loamy and clay in roach. The ratio of elementary soil particles (ESP) in soil content is a result of manifestation and interaction of soil-forming processes and physical weathering.
ESP distribution through the soil profile is given by sub-belts (Fig. 1). The minimum of the average index by the content of the sand fraction is typical of soils in subalpine meadows, but the variation of the index is maximum (Fig. 1a). The minimum variation of the sand fraction is noted in soils in crooked forest. The minimum value of the average content of coarse-silty fraction is typical of soils in crooked forest and the maximum of park woodland soils (Fig. 1b). Less index variation is noted for soil in subalpine meadows and greater for parkland soil. The minimum value of the average index for the clay fraction is typical of soils in subalpine sub-belt (Fig. 1c). The maximum variation of the index observed in the soils of parklands and the minimum in the soils of sparse forest.



a) b) c)

Fig. 1. Statistical diagrams (Box Plots) of distribution range of grain size fractions content: a) sand fraction, 100-50 mkm; b) coarse-silty fraction, 50-10 mkm; c) clay fraction, <10 mkm (crooked forest n=9, subalpine meadow n=16, parkland n=8)

Thus, the content and the ratio of ESP factions is different within the subalpine belt. Sand and clay fractions content is more stable in crooked forest soils. Subalpine meadows soils have a smaller variation of the clay fraction. In parklands, soils are characterized by diversity of all factions content.
ESP accumulation and removal throughout the soil profile varies in the Middle Urals. At higher elevations (920 m) in crooked forest there is a sharp reduction of the clay fraction content, while the the silt fraction content in the profile does not change and the sand and coarse-silty fraction increase down the profile (Fig. 2).

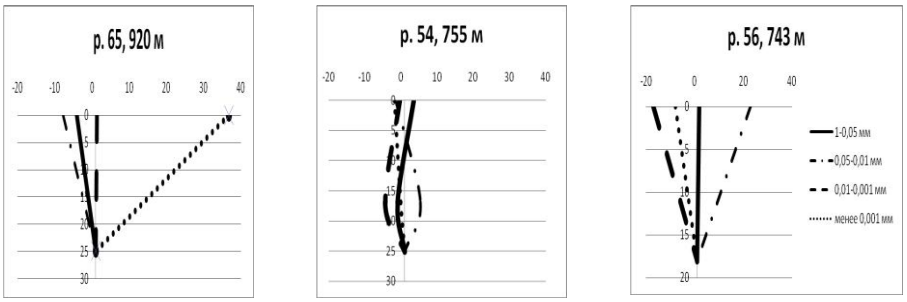


Fig. 2. Size fractions in grain size distribution of crooked forest soils

At an altitude of 750-800 m in the middle of the soil profile accumulation of coarse-silty fraction is marked. In litho-gley soils (c. 56) there is depletion of silt and clay fractions and a sharp increase of their content in roach. Thus, different elementary soil particles ratio in the grain size distribution is formed in crooked forest soils depending on the conditions of soil forming.

In subalpine meadows soils (Fig. 3) grain size fractions distribution in organic-accumulative soils (c. 69, 68, 63, 66) proceeds as follows: accumulation of silt and depletion of sand fraction in the upper horizons, followed by a decrease and an increase in roach, correspondingly. Organic-accumulative soil at an altitude of 700 m (c. 5) has the lowest content of coarse-silty and clay fractions.

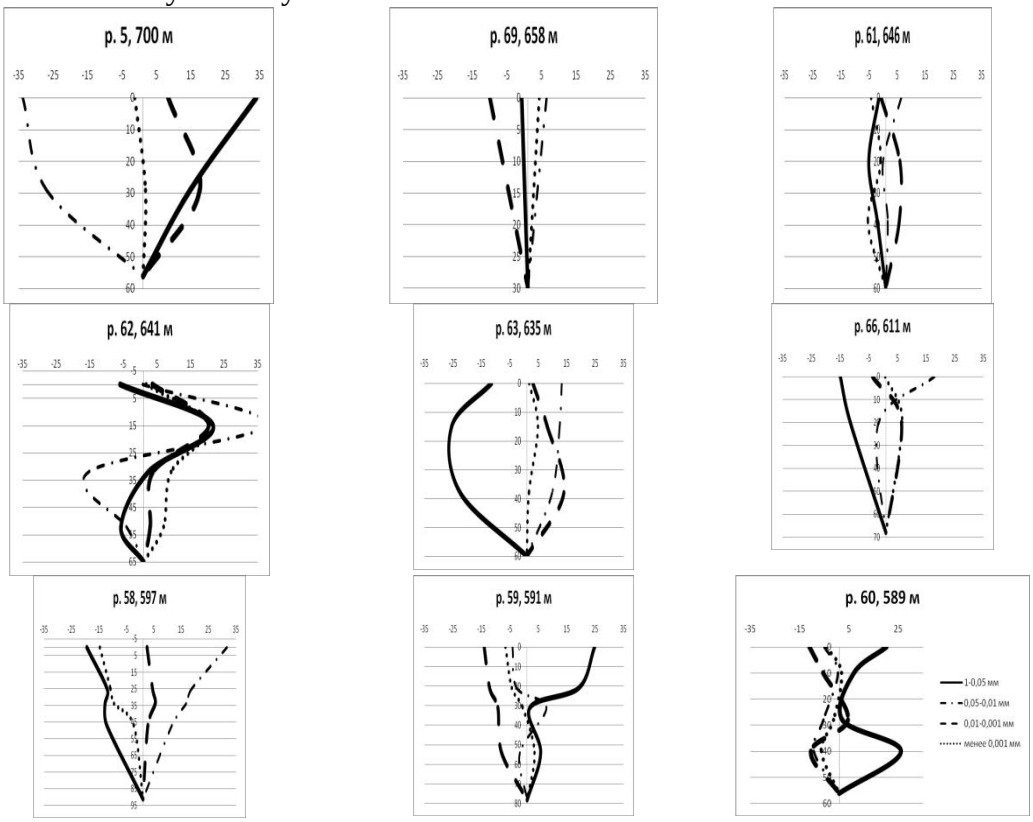


Fig. 3 Grain size fraction distribution in subalpine meadows soils

Fossil organic-accumulative soil at an altitude of 589 m (c. 60) has complex fractions distribution. In the humus horizon there is an accumulation of sand fraction, followed by a decrease in it, but in the fossil horizon [AY] there is an increase in sand content. The content of silt and clay fraction increases with depth, and in the horizon [AY] a reduction in the

content of these fractions occurs with a subsequent increase in roach. Thus, this ESP distribution leads to the conclusion that the soil is polygenetic.
In grey metamorphic soils, elementary soil particles content have a different ratio. So, in c. 61 there is a reduction in clay and sand fraction and an increase in coarse-silty and silty fraction, and in c. 62 all fraction increase and then decrease in roach.
In brown soils (c. 58, 59), more diversified ratio of the particles is observed and an increase of the clay fraction in roach occurs.
In organic-accumulative soils (c. 55, 73, 72) of parklands (Fig. 4) sand fraction content decreases and accumulation of coarse-silt fraction occurs. At an altitude of 570 m (c. 1) the soil is depleted with sand fraction, and clay fraction increases with depth, but then reduces again in roach. Clay-elluvial brown soil (c. 57) has a low content of clay fraction. Grey humus lithosoil is depleted with silt and clay fractions through the profile and, on the contrary, enriched with sand and coarse-silt particles.

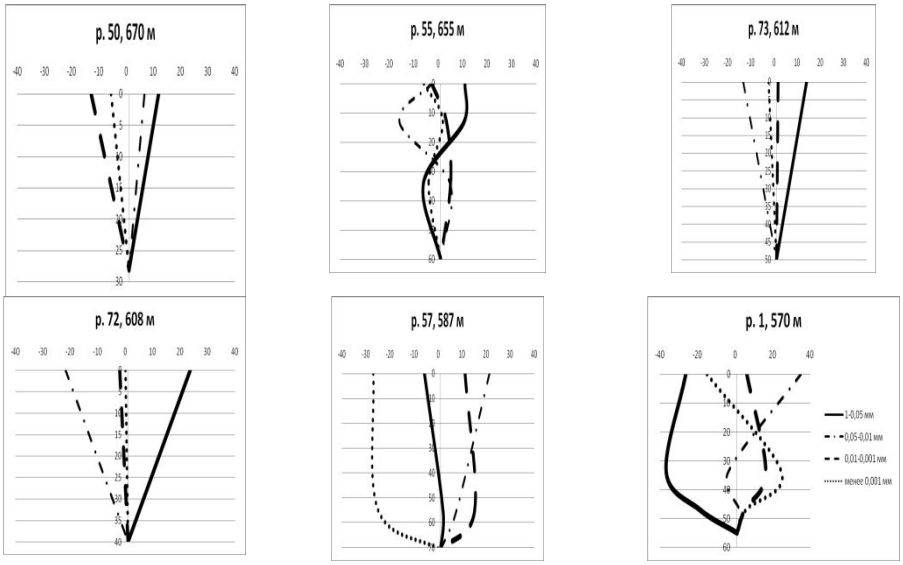


Fig. 4. Size fractions in grain size distribution in parkland soils

We have conducted a correlation analysis to determine the relationship between the content of GSD fractions and soil properties. Thus, in crooked forest soils contents of all fractions is primarily dependent on the altitude. In addition, the sand fraction content has a close inverse relationship with the profile thickness (r = 0.85). The content of the clay fraction has a very close relationship with the profile thickness and medium relationship with the soil rubbility, and the average inverse relationship with the altitude (r = -0.47).
In subalpine meadows sub-belt the content of coarse-silty fraction has an inverse strong relationship with the altitude (r = -0.73). The content of the clay fraction in soils of subalpine meadows is most strongly related to the profile thickness (r = 0.73) and average with soils rubbility (r = 0.45).
In soils of parklands, a close inverse relationship with the profile thickness observed for the silt and coarse-silt fraction, and the average relationship for clay and sand fraction. The content of silt fraction has close inverse relationship (r = -0.92) with area altitude, and sand fraction has an average inverse relationship with it.

Conclusion

Grain size distribution is an important soil diagnostic feature, because it helps to determine the degree of profile elluviality and illuviality. Soil profile differentiation by grain size

distribution is a result of regular changes in the activity profile of chemical and physical weathering, and relations between them. Location of soil at an altitude affects the grain size distribution due to different manifestations of denudation and steepness of the slope which affects the processes occurring in the soil and respectively the distribution of elementary soil particles.

The following features can be distinguished based on soil grain size in subalpine belt: grain size distribution is loamy; high content of coarse-silty fraction associated with the presence of physical processes of rocks and minerals weathering; distribution of coarse silt through the profile is very poorly differentiated; soil profile is mostly differentiated by the content of sand and silt fractions; ratio of elementary soil particles depends on the area altitude and vegetation at this altitude, as well as on the soil profile thickness.

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THE USE OF CRAB BY-PRODUCTS OF RAW CRUSTACEANS IN THE TECHNOLOGY OF RECYCLING OF RESOURCES IN AGRICULTURAL PRODUCTION

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Abstract

Chitosan is a promising component for modification of the prescription food due to functional-technological and bio-correction properties. Traditional resource base of chitosan - nibs from the shells of crab - has a high cost and limited resources. The evaluation of process efficiency in the use of alternative raw materials - shells of freshwater crayfish, shrimp, industrial waste recycling oyster mushroom for dietary chitosan. Technological parameters in assessing the effectiveness of various types of raw materials were the number of stages in the production of chitosan, the total duration of the process of chitosan and the output of the finished product. Chitosan from various sources received by traditional technology, comprising the sequential steps of deproteinization and demineralization of chitin-containing raw material obtained by deacetylation of chitin. The results show that for dietary chitosan to use the most technologically advanced crab by-products raw shrimp in comparison with other sources available in the regions remote from the pool of the king crab catch. The rational dosage of chitosan in compoundings of the meat chopped semi-finished products focused on a segment of the market of consumers with average level of the income is proved. It is shown that chitosan from an armor of a shrimp provides increase of functional and technological properties of mincemeat in the range of concentration of 1-4%. On the basis of an organoleptic assessment of products its dosage – 2% to the mass of meat raw materials is defined. Quality of the developed meat chopped semi-finished products with addition chitosan conformed to requirements of normative documents.

Keywords: *Chitin, chitosan, polymers, degree of deacetylation, functional properties*.

Introduction

Actual problem at the present stage of the development of engineering and technology is the safety of biological systems. This problem is directly linked to the health of the person in modern conditions, which are characterized by unfavorable environmental conditions over large areas, pollution from human waste, especially waste of polymeric materials.

The most important task facing the food industry - the preservation and maintenance of human health through nutrition. Another important task is to ensure the safety and sustainability of products and industries, which is to develop effective and efficient ways for processing of secondary resources sector of agriculture. Perspective raw material for the decision of tasks is a natural biopolymer – chitosan [Alieva, 2001; Antipova et al., 2004; Varlamov, 2002]. This polymer has many unique properties: high biological activity and compatibility with the tissues of humans, animals and plants, does not pollute the environment, because it is destroyed by enzymes of microorganisms, can be widely applied in environmental protection [Nikitenko et al, 2013; Slivkin et al, 2013].

Objective: to evaluate the technological efficiency in the use of alternative raw materials - shells of freshwater crayfish, shrimps, industrial waste recycling of oyster mushroom for production food chitosan and for its appropriate dosage with the composition of meat stuffing.

Materials and methods

We used crab shell containing raw material (SCRM) obtained in the industrial processing of freshwater crayfish, Arctic shrimp, as well as chitin-containing industrial wastes of mushrooms of the genus Pleurotus, meat minced systems based on beef and pork from only one variety of trimming, which was used as the basis for the development of modified recipes of meatballs and schnitzels and burgers and schnitzels culinary readiness with the use of chitosan in the range of concentrations from 1 to 4 % by weight of raw meat. The definition of functional and technological properties of multicomponent meat stuffing systems with chitosan, organoleptic and physico-chemical indicators of the quality of meat products culinary readiness conducted according to the recommendations [Antipova et al., 2004].

Mass fraction of moisture in raw materials and finished products was determined by drying the samples at 100-105 °C for 5 h [Antipova et al., 2004].

Mass fraction of protein in raw materials and products was determined by Kjeldahl method.

Mass fraction of fat in the raw materials was determined by the extraction method for defatted residue in accordance with the recommendations [Antipova et al., 2004].

Mass fraction of mineral substances was determined after combustion of organic matter in a muffle furnace at the temperature of 500-700 °C for 5-6 h to constant mass in accordance with the recommendations [Antipova et al., 2004].

For the isolation of chitin dry crushed raw material was mixed with water in the ratio 1:10, after which the mixture portion has made a concentrated hydrochloric acid at room temperature. At the stage of demineralization the pH change was controlled, at the increasing of pH to 7 the following portion of the acid was added. The demineralization process was considered complete at the pH 3-3,5, not changing for 30 min. It was established experimentally that the total amount of acid introduced at the stage of demineralization, corresponds to a solution of hydrochloric acid with a mass fraction of 4-4,5 %. The period of time during which the portioned acid was added amounted to 60-65 min. Then demineralized SCRM was washed with tap water to pH 6,5-7,0.

For removal of protein fractions (deproteinization) from demineralized SCRM we used the solution of sodium hydroxide with mass share of 5 % at 100 °C for 1,5 hours. Processed with alkali the chitin-containing raw material was cooled to ambient temperature and washed with distilled water to pH 7.0 at a water ratio of 1:6. The obtained chitin was washed and air-dried for 24 hours.

Then there was made the deacetylation of chitin. To dried chitin was added a sodium hydroxide solution with a mass fraction of 50 % and heated in a water bath for 2 h. The solid residue was separated from the liquid fraction by centrifugation and washed with distilled water to pH 7,0. The obtained chitosan was washed with an aqueous solution of ethanol with a volume fraction of 20 % and acetone until colorless, repeatedly washed with distilled water to neutral reaction and dried in air.

The quality of the obtained chitosan was adjusted on a complex of indicators. The content of minerals was established by dry ashing.

The degree of deacetylation was determined by potentiometric titration on universal ionometer EV-74 using a glass electrode.

The molecular weight of chitosan was determined by a standard viscometric method. The measurements were carried out at 250 °C in a capillary viscometer Ubbelohde, the diameter of which is equal to 0.54 mm. Calculation of molecular weight was carried out according to equation of Mark-Kuhn-Houwink [Gartman et al, 2013].

Residual protein in chitosan was determined by the colorimetric method with biuret reagent.

Functional and technological properties of model force meat with chitosan from crab by-products of raw shrimp were determined in accordance with the recommendations of [Antipova et al., 2004].

Results and discussion

The process of allocation of chitin and chitosan was preceded by a general analysis of the chemical composition of raw materials (table 1). The next stage of the study was the determination of the mass fraction of chitin in raw materials of animal and vegetable origin (table 2). Chitin refers to insoluble polymers, therefore, to its separation from the fruiting bodies of mushrooms, crabs and shrimp need a consistent and complete removal of protein and mineral parts of the material – deproteinization (DP) and demineralization (DM).

Table 1 - Total chemical composition of a crayfish shell, and mushrooms of oyster kind.

Object of investigation	Mass share, %				
	Moisture	Protein	Fat	Carbohydrate	Ash
Crayfish (SCRM)	64,54	11,43	0,45	-	13,97
Shrimp SCRM	73,43	8,37	2,12	-	8,98
Oyster mushrooms	89,22	1,67	0,52	6,78	0,81

Table 2 - Content of chitin in SCRM of crayfish and oyster mushrooms

Object of investigation	Mass share of chitin, %
Crayfish (shell)	9,61
Shrimp SCRM	7,18
Oyster mushrooms	0,52

The release of chitosan from various raw material sources was carried out in accordance with the traditional technological scheme which includes crushing stages of the initial raw material, deproteinization, demineralization and deacetylation of obtained chitin [Gartman et al, 2013]. Characterization of physicochemical properties of chitosans obtained by a general process scheme from various sources, illustrates the data presented in table 3. Physico-chemical properties of the obtained products of the deacetylation of chitin identified as meeting the requirements for food chitosan (JC-067-00472124). As it follows from the data presented in table 3, the chitosan from shrimp shell has physicochemical properties similar to the properties of chitosan of other crustaceans, forming in acetic acid solution with a mass fraction of 2% more viscous solutions, and also under the same conditions of obtaining isdistinguished by a higher degree of deacetylation.

Table 3 - Comparative characteristics of physico-chemical qualities of chifosan from different sources

Indices	Permissible meanings for food chitosan (TC 9289-067-00472124)	Sources of chitosan		
		SCRM of Shrimp	SCRM of crayfish fresh	Biomass of oyster mushrooms
Characteristic viscosity (in the 2 % solution of acetic acid), dl/g	Is not normed	11,56	10,7	10,15
Molecular mass, to	Is not normed	56,0	52,0	43,0
Degree of deacetitisation, %	Not less than 75	78	76	75
Ash,%	Not more 0,7	0,4-0,45	0,4-0,5	0,1
pH of 1 % solution in 2 % of CH_3COOH	Not more 7,5	5,9	6,0	6,5
Protein residue, %	Not more than 0,1	0,05-0,1	0,01-0,05	0,04
Mass share of moisture, %	Not more than 0,1	9-10	8-10	8-9
Appearance	Scales size 1-3 mm	Scales size 1-2 mm	Scales size 1-2 mm	The size of particles 0,1-0,2 mm
Bulk mass	Is not normed	0,25-0,4	0,15-0,4	0,6-0,7

These functional properties of chitosan from SCRM shrimp allow to consider it as perspective functional-corrective component of food products. The introduction of chitosan into the water, which does not interact chemically with it, provides a rapid swelling of the polymer, and every bit of chitosan swells separately from the others and the resulting mass (Sol) is homogeneous (free of lumps).It was established that chitosan from shrimp shell has a fast swelling properties: water absorption was 10,9 cm^3/g for 4-5 minutes. The fact that chitosan of shrimp SCRM is a hydrocolloid, determines its potential functionality processing for food purposes of livestock products in branches of meat, fish and dairy industries.

Technological efficiency in the use of SCRM of crawfish, shrimp, industrial waste recycling of mushrooms of the genus Pleurotus for food chitosan was assessed by the following indicators: the number of stages in the production of chitosan, the total duration of the process of obtaining chitosan and the product yield (table 4).

Table 4 - Evaluation of technological efficiency of different kinds of raw materials for obtaining of chitosan

Technological parameters	Kind of raw materials		
	Shrimp	Crayfish	Mushroom
Quantity of stages chitosan production	3	4	5
Total time of chitosan production, h	5	6,5	7
Output of chitosan, % to mass share of the raw material	5,03	4,91	0,45

The results indicate that for the production of food chitosan is technologically better to use SCRM shrimp in comparison with other sources. This is actual for regions that are geographically distant from traditional and new basins of the king crab catch.

Testing of chitosan was performed in the concentration range from 1 to 4% for multicomponent forcemeat systems for meatballs and schnitzels with a ratio the price – quality oriented on consumers with average income. It was investigated the effect of chitosan on functional and technological properties of meat model systems – water-binding capacity (WCC), water-holding capacity (WHC), fat-holding ability (FHA), emulsifying ability (EA) and emulsion stability (ES). In model meat systems we used the stuffing from one-grade beef and pork chopping on the mincer with a hole diameter of the lattice 2-3 mm at the following ratio: 2:1 for meatballs, 5:4 for schnitzel.

It was established that chitosan improves functional and technological properties (FIP) of meat systems. Values of the FIP model stuffing for meatballs and schnitzels had no significant differences. The nature of the effect differed for individual indicators. So, the WCC has increased by 20% on average for the tested systems at the concentration of chitosan 2 %, further increase did not give significant effect. Increase WBC was 14,5 %; stabilization of this indicator occurred at 3 % of chitosan. FHC increased by 21,7 and 22,7 %; EA – 26,05 % 23,11 % for model force meat of for meatballs and schnitzels, respectively, when the mass fraction of chitosan 4 %. Similar curves were obtained for emulsifying ability, it increased by 26,1 and 23,1 %.

In the next phase of research were defined organoleptic characteristics of molded film from model force meat of meat products after cooking. The addition of chitosan in the amount of 1 % does not affects the organoleptic characteristics, 3 % leads to a slight deterioration of taste, and the dose increased to 4 % leads to considerable deterioration.

Conclusions

The established identity of the physico-chemical properties of chitosan, obtained under similar conditions from SCRM of shrimp and freshwater crayfish, testify to their similar chemical nature and molecular structure. Chitosan obtained from mushrooms of the Pleurotus genus, is distinguished by the following characteristics: intrinsic viscosity (in 2 % acetic acid), molecular weight, degree of deacetylation, primarily due to the formation of the chitin, the chitin-glucan complex (CGC).

Technological indicators characterize the chitosan from shrimp SCRM as polymorphic system with a dominant fraction of particles of 1-2 mm, a moisture content of not more than 10 %,

with poor flow ability and a very small bulk mass.
The viscosity of chitosan solution in acetic acid solution with a mass fraction of 2 % and the ability to swell in aqueous environments are considered as a perspective functional food component, including for the prevention of alimentary-dependent diseases.
Thus, SCRM shrimp can be processed in conditions compatible with conditions of food, including fish processing enterprises with obtaining food chitosan in the form of additional marketable products. Using of chitosan in the amount of 2 % by weight of raw meat does not deteriorate the organoleptic characteristics of the products. It can be used to obtain a wide spectrum of meat and food products, with high quality and mass yield. The content of chitosan in an amount of 2 g per 100 g of the product is 40 % of the adequate level of consumption of chitosan per day, and allows to meet the daily requirement of dietary fiber fiber on 10 % [Rosstat, 2015]. Such products can be recommended for people with obesity, with a lack of function of the digestive system, diseases of the pancreas; for elderly people.

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BUFFERING PROPERTIES OF MOUNTAIN SOILS TO ACID EFFECTS, AND THEIR ABSORPTION CAPACITY

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Abstract

Mountain Soil in subalpine zone of the Middle Urals formed in specific environmental conditions associated with the mobility of rock debris on the slopes, a failure in intensive filtering the wash water regime, a significant number of herbaceous litter. A specific feature of these soils is the high acidity: pH salt extracts slightly vary within 3.7-4.2, indices hydrolytic acidity 10-22 mEq / 100 g. Buffer properties of soils were determined by continuous potentiometric titration of salt suspensions prepared in ratio of 1: 2,5. According to the results of potentiometric titration determined by calculation methods the number of protons absorbed. Graphical methods helped to calculate buffer area and the degree of reduced buffer capacity. The pH dropped to an average of 2,50-3,16; ΔpH figure was 0,9-1,45. The amount of absorbed protons averaged 70-90 mg / kg of soil, which corresponds to 75-95% of the amount added. In general, the buffer properties of the soils in relation to acid are low. This is confirmed by the reduced buffer capacity indicator constituting 7-27% of the reference area with absolute buffering capacity. This figure corresponds to very low and low buffer capacity of soils to acid attack.

Keywords: *mountain soils, buffer, protons, absorption, acidity*

Introduction

Acid-base buffering capacity is one of the measures of soil adsorption capability. Studying of acid-base buffering capacity of soils is of great theoretical and practical significance in connection with the progressive acidification of soils in some regions of the world due to both natural and anthropogenic factors. Studying of buffering capacity is recommended in order to gather data on modern soil formation and soil genesis (Zayzheva, 1987; Savchenko, 1989; Ponizovskiy, Pampura, 1993; Pevnuy, Sokolova, 1997; Nadtochiy, 1998;, ,Shamrikova et al., 2002; Shamrikova et al., 2003; Ufimtseva, Pokatilova, 2005; Ushakov, 2005; Tolstokaeva, Zharikova, 2009; Shamrikova et al., 2011; Shamrikova, Sokolova, 2013; Soil and soil cover…, 2013; Lyzhenko et al., 2014).

Most of natural soils in humid regions of the taiga-forest zone is characterized by acid pH and low bases saturation (Podzol Soil…, 1980; Podzol Soil…, 1981; Forest Soil…, 1997). This is a wide range of soils: podzolic, sod-podzolic, sod-gley, brown forest, swamp-podzolic, glebous swamp. In addition to the natural acidic background of forest soils, there is a source of anthropogenic acidification: acid rain. Most of researchers believe it is one of today's most important regional problems (Acidification today and tomorrow, 1982; Wisniewcki, Keitz, 1983; Tamm, David, 1989; Acidification research…, 1991; Derome, 1991). The problem of acid rain is related to the phenomenon of transboundary transport of acid-forming pollutants from industrial regions of Central Europe, as well as air pollution from its own sources of emissions (Makarov et al., 1994). Acidic solutions have both direct and indirect impact that is reflected in imbalances in elements intake by plants due to different intensity of their desorption into soil solution under the influence of acid rain (Sokolova et al., 2012).

The main mechanisms of buffering reactions are studied mainly using potentiometric titration (continuous potentiometric titration – CPT). The chemical reactions that cause buffering are

very diverse and include the interaction of H + and OH- ions with solid components of the soil body and the components of soil solution (Filep, Radley, 1989). Each soil type is characterized by its own set of mechanisms of such interactions. The effectiveness of acid-base buffering capacity is determined by the capability of soil systems to extinguish high amplitude activity of protons in reactions in soil according to the scheme: acid → base+proton (Nadtochiy, 1998).

Investigation of acidity and cation exchange properties being the most informative indicators of forest soils condition is an important part of their monitoring (Grishina et al., 1991; Makarov et al., 1994; Kopzhuk et al., 2001). This paper is particularly relevant for the soils of protected areas because of the specific characteristics and objectives of their functioning (Kopzhuk, Livantsova, 2003). Protected areas preserve habitat forming functions and biodiversity of ecosystems (Dobrovolskiy et al., 2003). In addition, the safety of PAs themselves depends on the state of the environment and its protection both in neighbouring and fairly remote areas. PAs can compensate for adverse anthropogenic impact since nature is capable of autoregeneration.

There are two major national parks in Perm region with protected natural forests of the Middle and Northern Urals. Studies on the soil cover of the Urals began later than in other mountain areas and were neither permanent or systematic and isolated (Samofalova et al., 2014). The most unique mountain landscapes in the Urals are believed to be those of the sub-alpine (subgoltcy) belt, where the mountain soils are formed under special environmental conditions associated with mobility of debris on the slopes, a collapsing filtering at intensive percolative regime, and a significant number of herbaceous litter (Samofalova et al., 2013, 2014). The high acidity is a specific feature of the soils, and therefore they are unstable for potential acid effects caused by industrial centers of Perm region. From this point of view, it is important to study the acidic properties of these soils and their buffering capability in relation to acid effects.

Materials and methods

The subject of the research are subgoltcy belt soils on Basegi mountain range located between 58^{o} 50' and 60^{o} N, to the west of watershed of the Urals, a part of the "Basegi" State Natural Reserve. This territory is located in an area of ridge-residual low-hill terrain in Middle Urals. The meridionally elongated range consists of three mountains: North Basegi (951.9 m), Middle Basegi (994.7 m) and South Basegi (851 m). Cold and wet climate with continental features. According to zonal distribution of vegetation, the area is located in the middle taiga subzone of boreal forest zone and the following belts can be isolated here: mountain forest, subgoltcy (subalpine) and mountain-tundra (goltcy). Subalpine belt is mild and includes three subbelts: parkland, subalpine meadows, and crooked forest.

The reserve is located far from industrial centres. There are Kizel-Gubakhinsky and Lysvensky-Chusovskoy industrial centres within 42-73 km to the west of the crest with a developed mining, chemical and metallurgical industries which are the sources of pollution and since the prevailing wind direction is west and south-west the air transport of pollutants in the protected area is possible.

Soil cross sections were made in subgoltcy belt on the North Basegi and Basegata mountains. Russian soil classification of year 2004 was used. Analytical studies were performed at the Department of Pedology of the Perm State Agricultural Academy. Buffering properties of the soils were determined by CPT of salt suspensions prepared at a ratio of 1:2.5. The titration was performed with 0.1 n solution of HCl; the total amount of acid of 10 ml corresponds to a load of 100 mmol/kg soil. Time of reaction of the acid with the suspension was 2 minutes. Based on CPT results, the number of absorbed protons was calculated; buffer areas and the extent of the reduced buffering capacity were calculated graphically. Based on the graphical

analysis, the buffer area was determined, as well as the reduced rate of buffering capacity, which is determined relative to the absolute buffering capacity set as 100%. The graph of buffering capacity of the sample looks like a horizontal line with pH = 7. The scales given in (Glazovskaya, 1990; Bogdanova, 1994; Nadtochiy, 1998) were used to estimate soils buffering capacity.

Results and discussion

Soils have a truncated profile mildly differentiated on horizons with a high content of gravel. The investigated soils belong to the stem of postlitogenic soil forming. Based on morphogenetic characteristics of soils, the following formations were identified: lithosoil (layer thickness less than 30 cm), organic-accumulative (median horizon is not pronounced as an independent genetic formation), structural and metamorphic (picked horizon BM) (see Table 1).

Table 1. The natural conditions of soil formation

Cross section number, alt, m; E, G, soil name	Horizons	PT, cm	Vegetation, relief
North Basegi mountain			
C. 54, 755, south, 20, Gray humus lithosoil	AY_1-AY_2-AY_3	17	Birch crooked forest; moderately drained levelled area
C. 49, 617, southern, 3-5; Organic-accumulative	AY-Aym-CLM	40	Miscellaneous grass and gramineous meadow; slightly drained levelled area
Basegata			
C. 62, 641, western, 5, Grey metamorphic	AY-AEL-BM_1-BM_2-CLM	65	Silk and miscellaneous grass meadow; drained gentle
C. 58, 597, western, 5, Elluvial brown soil	AY_1-AY_2-AYel-BM-CLM	91	John's-wort and miscellaneous grass meadow; drained gently sloping area
C. 60, 589, western, 5, Fossil, organic-accumulative	AY-AYm-AY(CLM)-[AY]-[CLM]	56	Silk and miscellaneous grass meadow; slightly drained gently sloping area

Note: E – slope exposition; G – slope gradient; PT – profile thickness.

Actual acidity in soils is characterized by pH of 4.8-5.6. The higher pH value in cross section 54 is probably related to the nature of plant litter in birch croocked forest where base saturation is higher. pH values of salt extracts vary from 3.5 to 4.2 within the profile. Hydrolytic acidity values are 10-22 mEq/100 g. Soil absorbing complex is significantly not saturated with bases, in some cross sections the degree of unsaturation reaches 80-90%. Biological accumulation of bases does not compensate for their removal with the groundwater flow, although some reduction in the degree of unsaturation has been noted in the humus horizons.

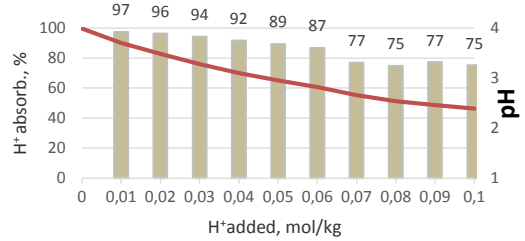
The CPT method allows to register mostly fast proceeding buffering reactions, which include ion exchange, protonation and deprotonation of pH-dependent exchange positions and dissociation of organic and mineral compounds in the solid body. Based on CPT results, the number of absorbed protons was calculated with the following formula:

$H^+absrpd. = H^+src. + H^+add. - H^+fin.$,

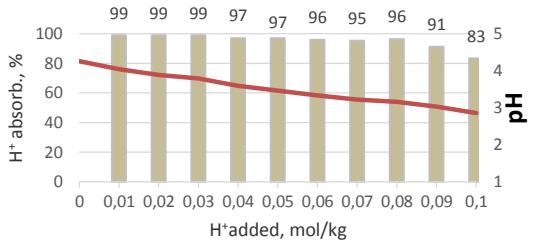
where $H^+src.$ is the source number of H^+ protons in suspension; $H^+add.$ is the added number of protons; $H^+fin.$ is the final number of protons in suspension after reaction with the solid body of the soil.

The pH of soil suspension decreased to an average of 2.50-3.16 after adding of the maximum amount of acid (Fig. 1). In the acid exposure on the first (top) humus horizons, pH shifted 1.1-1.3 units relative to the initial values. In the lower humus horizons, ΔpH was 0.90-1.45. The highest pH value noted in grey humus lithosoil (c. 54) with less apparent acidic properties. According to scale (Glazovskaya, 1990) such ΔpH value means that buffering capability of

mountain soils is above average. Lowering the pH results in the soil properties deterioration, as aluminum and iron ions at concentrations toxic to biota emerge in the soil solutions.
In humus horizons, ΔpH largely depends on the level of hydrolytic acidity (r = -0.85), content of clay (r = -0.89) and silt (r = -0.83) fractions, and also on removal of the silt fraction (r = -0.92) and accumulation of the sand fraction (r = 0.65), as well as conservative part of organic matter content (r = -0.71).
The amount of protons absorbed at the end of titration averaged 70-90 mmol/kg of soil, which corresponds to 75-95% of the amount added. The absorption capacity decreases as the proton concentration in the solution grows. In suspensions from humus horizons it decreases from 97 to 70%. Increasing the intensity of the absorption observed at pH below 3.9-3.6; 3.0; 2.5. The absorption of protons in these pH ranges is a result of reactions of displacement of exchange bases, dissociation of organic and mineral complexes followed by protonation of organic ligands, mineral dissolution.

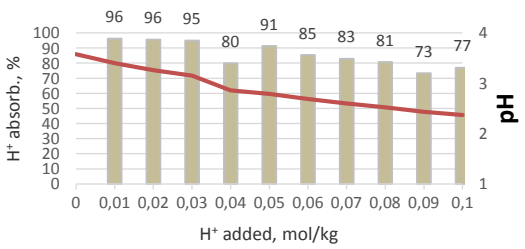


a) AY_1

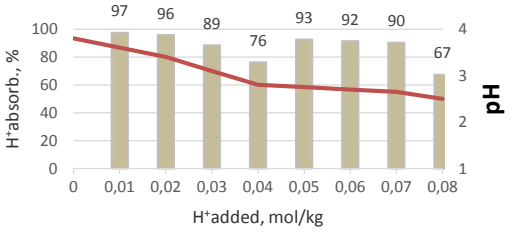


b) AY_2

Gray humus lithosoil (c. 54)

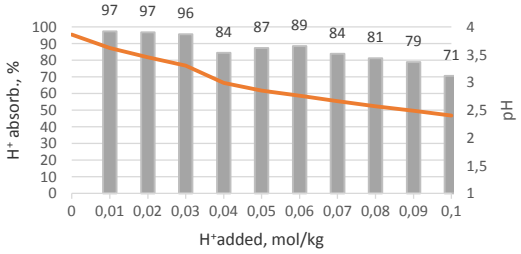


c) AY

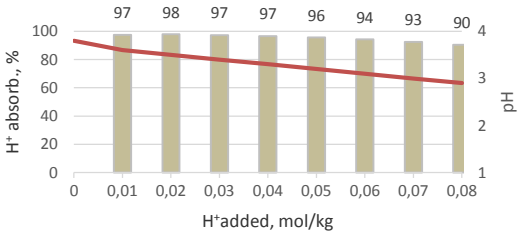


d) AEL

Grey metamorphic (c. 62)



g) AY_1



e) AY_1

Elluvial brown soil (c. 58)

Figure 1. Change in the adsorption capability and pH at the acid titration of soil suspensions of humic soil horizons in subalpine belt

In general, these areas are characterized by high capacity, as indicated by ΔpH (see Table 2). The buffering area is low which indicate that the soil "buffers" mildly. The buffer capacity was 7-27% of the area of the sample with absolute buffering capacity, which corresponds to a

very low and low buffer capacity of soils to acid effects. It should be noted that buffering capacity of mountain soil to acid effects decreases along with altitude decrement (r = 0.76).

Table 2. The buffering area and the degree of reduced buffering capacity of mountain soils

Cut №	Horizon, depth, см	pH CPT	The buffering area, sm^2	The degree of reduced buffering capacity	ΔpH
54	AY_1 2-9	4,3	9,0	27,0 (low)	1,4
49	AY_1 5-14	3,7	6,0	20,0 (very low)	1,2
62	AY	3,6	7,0	22,0 (low)	1,2
58	AY_1 2-25	3,9	6,0	19,2 (very low)	1,5
	AY_2	3,8	4,3	15,8 (very low)	1,2
60	AY	3,3	1,1	6,8 (very-very low)	1,9

Reduced buffering capacity is largely dependent on the content of alluvium (r = 0.73), medium and fine dust (r = 0.53), and also on the accumulation of dust (r = 0.82) and removal of the sand fraction (r = -0.56). Buffering properties of soils in subalpine belt have an average dependence on the silicon content (r = 0.46) and inverse dependence on the iron content (r = -0.68). Thus, buffering properties are associated with mineralogical composition of the soil as indicated by the relationship between reduced buffering capacity and the entropy of the bulk composition (r = -0.70). At the most non-uniform distribution of the system components, entropy tends to zero thereby increasing the buffer soil resistivity. The reduced buffering capacity has a direct relationship with the content of organic matter (r = 0.76) and its conservative part (r = 0.64), and a reverse relationship with a degree of humification (r = -0.67).

In the studied humus horizons, buffering intensity peaks are well defined in the pH range of 2.7-2.45. At the same time the pH values are consistent with humus content. Obviously, the buffering reactions in this range are associated with organic and mineral derivatives of humic acids.

Decrease of the solution pH causes a decrease in negative charge of organic compounds due to attenuation of ionization of COOH groups and reducing the protonation of clay minerals in soils. Scientists believe that at recharge of colloidal particles of $Fe(OH)_3$ and $Al(OH)_3$ and organic colloids of amphoteric nature their positive charge also increases (Motuzova, 1994). As a result, base exchange capacity, strength of bond between metal and soil and selectivity coefficient decrease, which in turn results in elevated proportion of metals in soil adsorption complex, so elements adsorption decreases in general.

20 to 80 mmol/kg acid we spent on suspension titration in pH fange 2.7-2.45. Among the analyzed soils structurally metamorphic soils had the maximum buffering capacity to the acid: elluvial brown soils (c. 58), grey methamorphic (c. 62). Organic-accumulative soil and grey humus lithosoil show less resistance to acid.

Conclusion

Increasing the acid load of mountain soils in the subalpine belt reduces their adsorption capability. Thus the soils of mountain landscapes are sensitive to acid effects. In the buffering structure, pH values of 3.9-3.0 and 2.5 are thresholds. In pH interval from the start point of titration to 3.0 in all horizons the main buffering reaction is displacement of exchangable cations by a proton. In the horizons containing organic material the dissociation reaction of organic and mineral complexes is added to them followed by protonation of organic ligands. At lowering pH below 2.5 units dissolution of iron sesquioxide occurs. Reactions associated with dissolution of mixed-layer clay minerals are possible in mineral horizons (AEL).

Soils in subalpine belt have low and very low buffering capacity and as a consequence low environmental and geochemical resistance to acid effects. Reduced buffering capacity is the

most objective indicator to evaluate the buffering properties that allows to compare soils formed under different environmental conditions. At the same time the use of the relative pH shift to assess buffering capacity is justified for soils with similar acidity values. Evaluation of buffering capacity and absorption capability of soils can be used as an objective criteria for qualitative assessment of the soil state for soil and environmental monitoring.

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IMPACT OF THE CONTENT OF MOBILE PHOSPHORUS AND POTASSIUM ON BIOLOGICAL ACTIVITY OF THE SOIL

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Abstract

The studying of the soil biological activity is one of the most important criteria for evaluating the direction of soil processes. Biological activity of the soil allows about a condition of fertility of the soil. Microbiological activity depends on the type of soils, properties and many other factors. Anthropogenic loading in bigger or smaller intensity can cause suppression of the functions which are carried out microorganisms that leads to violation of their activity. In Perm Krai sod-podzolic soils of heavy granulometric composition occupy 69.6 % of the area of an arable land. Features of these soils – sour reaction of the environment, the relative poverty in elements and organic substance, is poorly expressed by structure, the availability of the inert podzolic horizon, and also flushing type of the water mode. All it determines the low level of biological activity of this type of soils. We have investigated the change in the biological activity of sod-podzolic soils with different content of available phosphorus and potassium. Mathematical processing of experimental results testifies the direct dependencies of content of mineral nitrogen in the soil on the content of available phosphorus and potassium (r = -0.415-0.952). Studies have shown that the content of mineral nitrogen in the soil depends on the content of labile phosphorus and potassium, which suggests an ability to predict the future availability of nitrogen for crop seeds. The best conditions for nitrification are marked on variants A_2B_1 (80.1 mg NO_3^-/kg/14 days) and A_3B_2 (77.0 mg NO_3^-/kg/14 days), worst on A_1B_1 (54.4 mg NO_3^-/kg/14 days) and A_3B_1 (51.3 mg NO_3^-/kg/14 days). Sod-podzolic soils of heavy granulometric composition have low biological activity, but when creating the optimal conditions for microbial, their activity increases.

Keywords: *Sod-podzolic soil, biological activity, phosphorus, potassium*

Introduction

The studying of the soil biological activity is one of the most important criteria for evaluating the direction of soil processes and allows to judge about change of the nitric mode of soils. Anthropogenic impact on soil not only can cause suppression of microorganisms functions, which leads to violation of activities of the latter, but can also improve the conditions for the occurrence of positive microbiological processes in the soil (Mudrykh et al., 2013, 2014; Kizilkaya et al., 2012, 2013).

Long-term studies established that the majority of arable crops most vigorously consume nitrate nitrogen from the soil. Scientists attribute this to the fact that the intake of nutrients (in the form of cations) into the plant is greater at the nitrate assimilation and to change in the ratio of ammonium and nitrate at the cultivation of the soil. Improving the physical and chemical properties tends to raise the proportion of nitrate in mineral nitrogen reserves: from 20 % in soils with low fertility to 60 % in well-cultivated soil. Nitrogen fertilization increases this number to 90 %, which is due to the intensification of nitrification in soils. Creating optimal conditions for the cultivation of arable crops improves conditions for the nitrification process, as these conditions are similar. So that is not a coincidence that the high nitrifying

activity has long been used as an indicator of good soil fertility (Koren'kov, 1990, Kidin, 2008).
The purpose of the study is to determine the effect of labile phosphorus and potassium on the soil nitrification process.

Materials and methods

Perm Krai is situated in Western Predural'e region and defined by 56°06′-61°39′ of northern latitude and 51°47′-59°03′ eastern longitude (Fig. 1). From north to south the territory of the Krai stretches for 600 km, from west to east in the southern part of the Krai – for 200 km, in the northern part – for 500 km (Ashkin et al., 2012, 2013: Mudrykh et al., 2014).

Figure 1. Location of the experimental field in Perm, Russia

The soil samples (56) was taken from experimental field at the Experimental Station of Perm State Agricultural Academy, Perm, Russia (57°56′00″N, 56°14′59″E) in 2011.
A composite surface soil sample from 0-20 cm depth was collected from the experimental site before initiating the experiment and analysis of soil samples on agrochemical indicators was carried out by standard techniques (Peterburgskii, 1981). Soil samples were air dried at room temperature; sieved with < 1 mm screen. An experiment was conducted to determine the effects of available phosphorus and potassium on soil enzyme activities under laboratory conditions. The laboratory experiment was carried out according to the procedure described by Kravkov.

Table 1. Scheme of laboratory experiment carried out to Petrburgskii (1981)

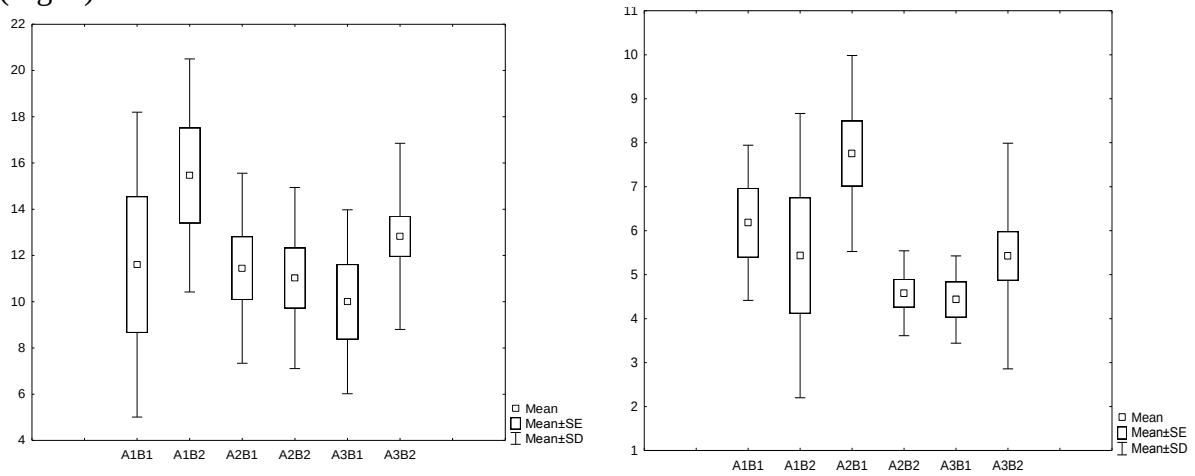
Factor B: Soil potassium range	Factor A: Soil phosphorus ranges (mg kg^{-1})		
	A_1 (101-105)	A_2 (151-200)	A_3 (over 200)
B_1 (41-80)	A_1B_1	A_2B_1	A_3B_1
B_2 (81-120)	A_1B_2	A_2B_2	A_3B_2

There was a double replication of the experiment variants. The experiment lasted for 14 days. Levels of labile phosphorus and potassium in the soil were formed artificially in experiments performed by the staff of the Department of Agricultural Chemistry with the use of organic and organic-mineral fertilizers. Analysis of soil samples for agrochemical parameters was carried out according to the standard procedures (Arinushkina, 1961; GOST, 1991, 1994; Mineev, 2001). The intensity of the nitrification was measured by the difference between the content of nitrate in the soil before and after incubation.
Soil samples have subacid pH close to neutral (5.3-5.6). Total exchangeable bases are within 18.2-20.2 mg-eq./100 g of soil. The soil is base-saturated (88-89 %), content of phosphorus 112.3-328.3 mg/kg, potassium 57.3-116.0 mg/kg of soil. Deviation of physic-chemical parameters values for variants of the experiment are within analytical error. Analysis of soil

samples taken before experiment beginning has shown that in major agrochemical parameters the soil in the experiment variants differ only in the content of phosphorus and potassium.
All data were analyzed using statistical software STATISTICA 7.0 and MINITAB 14.

Results and discussion

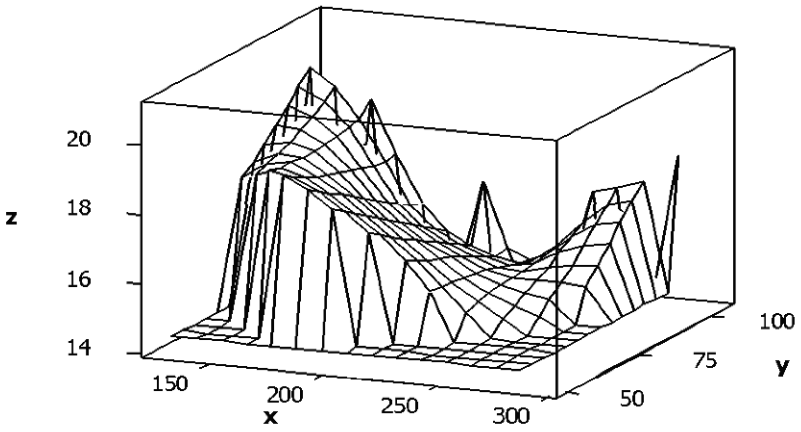
Petersburgsky (1991) has established that, in the non-chernozemic zone, 50 % in obtaining arable crops yield is due to nitrogen. Such a high increase in yield is due to the fact that the flushing regime of sod-podzolic soils, acid medium reaction, and the relatively low amount of organic matter determine low levels of nutrients in the soils of this type and, in particular, nitrogen. The content of mineral nitrogen in the soil depends on many factors. To improve the plants nitrogen supply, it is necessary to determine the mineral nitrogen content in the soil at certain stages of plant development to allow fertilizing, if necessary. Optimal conditions of nitrogenous nutrition of plants can be accomplished in two ways: soil cultivation (increasing reserves of nitrogen, controlling its transformation in soil) and application of mineral nitrogen fertilizers. The first one is the cheapest way to provide plants with nitrogen, as it requires no additional expenses and the method is environmentally safe (Koren'kov, 1990).
Soil samples were taken to determine the amount of mineral nitrogen in the soil in the tillering stage of winter rye before fertilization. Analysis of soil samples showed that the content of mineral nitrogen in the soil is very low (Fig. 2, 3). The maximum content of $N\text{-}NH_4$ (13.5 mg/kg of soil) was observed in variants with an average content of labile phosphorus (Fig. 2).



Figures 2. Impact of mobile phosphorus and potassium on the content of amount nitrogen (left) and nitrate nitrogen (right) in the soil, mg/kg

The increasing of labile phosphorus is followed by decreasing of amount of $N\text{-}NH_4$ up to 11.2-11.4 mg/kg of soil. Elevation of labile potassium level from low to average has led to a 2.1 mg/kg of soil increase of studied index. Detailed analysis and mathematical treatment of the results showed that the greatest range of variability of ammonium nitrogen was observed in the A_1B_1 variant, the lowest in A_3B_2. On variants A_1B_2 and A_2B_1 a close correlation between the content of labile phosphorus and ammonium nitrogen was established: r = -0.555 and -0.710. It should be noted that the amount of ammonium nitrogen in the soil is less dependent on the content of labile phosphorus, and more on potassium. The increase of potassium in the soil is followed by some increase in ammonium amount.
The content of nitrate nitrogen in the soil, as well as ammonia, is very low (Fig. 2).
On a variant with average content of labile phosphorus the amount of nitrate nitrogen was 5.8 mg/kg soil. An increase of labile phosphorus to high level was followed by slight increase of nitrate nitrogen content up to 6.2 mg/kg of soil. A further increase in the level of phosphorus in the soil led to some reduction in nitrate concentrations up to 4.9 mg/kg of soil.

In contrast to ammonium nitrogen, increase of the amount of potassium in the soil was followed by decrease in nitrate nitrogen amount. Thus, on variants with low potassium level the content of $N\text{-}NO_3$ was equal to 6.1 mg/kg of soil, and with the average level to 5.1 mg/kg of soil. Closer examination of the data indicated that the maximum content of nitrate (7.8 mg/kg of soil) is on the variant with a high labile phosphorus and low potassium content.
Mathematical analysis showed that the greatest range of variability of the ammonium nitrogen is on variant A_1B_2, the lowest on A_2B_2. With the increase of labile phosphorus in soils, in contrast to ammonium nitrogen, nitrate concentration decreases slightly. It is worth noting that the close correlation was noted on variant A_1B_1 between the content of labile phosphorus and nitrate nitrogen: r = -0.952. A similar dependence was observed in variants A_2B_2 and A_3B_1, the correlation coefficients were -0.517 and -0.415, respectively.
Evaluation of plants nitrogen nutrition was carried out on the basis of the content of mineral nitrogen in the soil ($N\text{-}NH_4$+$N\text{-}NO_3$). Comparison of mineral nitrogen accumulation in the soil in the tillering stage at different levels of labile phosphorus and exchangeable potassium shows that the Impact of the studied factors is not clear (Fig. 3).



Figures 3. Impact of mobile phosphorus (x) and potassium (y) on the content of mineral nitrogen (z) in the soil, mg/kg

Thus, at an average labile phosphorus level the maximum accumulation of mineral nitrogen up to 19.3 mg/kg of soil was observed. Increased labile soil phosphorus causes a reduction of the content of mineral nitrogen to 16.3-17.4 mg/kg of soil. Changing of labile potassium level in the soil from low to medium provides some increase in studied index from 17.1 to 18.2 mg/kg of soil. According to the providing group by the soil mineral nitrogen in the spring the amount of nitrogen in the experiment relates to class III, i.e. average. This indicates that mineral nitrogen level is sufficient for the initial development and growth. But for subsequent periods it is necessary to fertilize with additional nitrogen.
So, field data indicate a low biological activity in the soil, as the accumulated amount of nitrogen is not enough for the entire growing season, and additional expenses for feeding with nitrogen fertilizers are required for normal growth and development of winter rye.
Let's see if optimal conditions for biological activity will allow to achieve a sufficient, for winter rye, level of mineral nitrogen in the sod-podzolic soil.
To determine the intensity of the nitrification process under optimum conditions, was determined a content of nitrogen before and after incubation in samples for options of laboratory experiment. Analysis of the intensity of nitrification in sod-podzolic heavy loamy soil showed that the maximum microbial activity is observed in variants with a high content of labile phosphorus and medium content of potassium. On the variant with a high content of

labile phosphorus in the soil, nitrifying activity was 70.4 mg NO_3^-/kg of soil for 14 days. Decrease in labile phosphorus to an average and increase to high level led to a decrease in activity, respectively, to 59.7 and 64.2 mg NO_3^-/kg/14 days. Increasing potassium level from low to medium provided some increase in nitrifying activity from 61.9 to 67.6 mg NO_3^-/kg/14 days. The best conditions for nitrification are marked on variants A_2B_1 and A_3B_2, worst on A_1B_1 and A_3B_1.

Creation of optimal conditions for nitrification causes some accumulation of mineral nitrogen (Figure. 4).

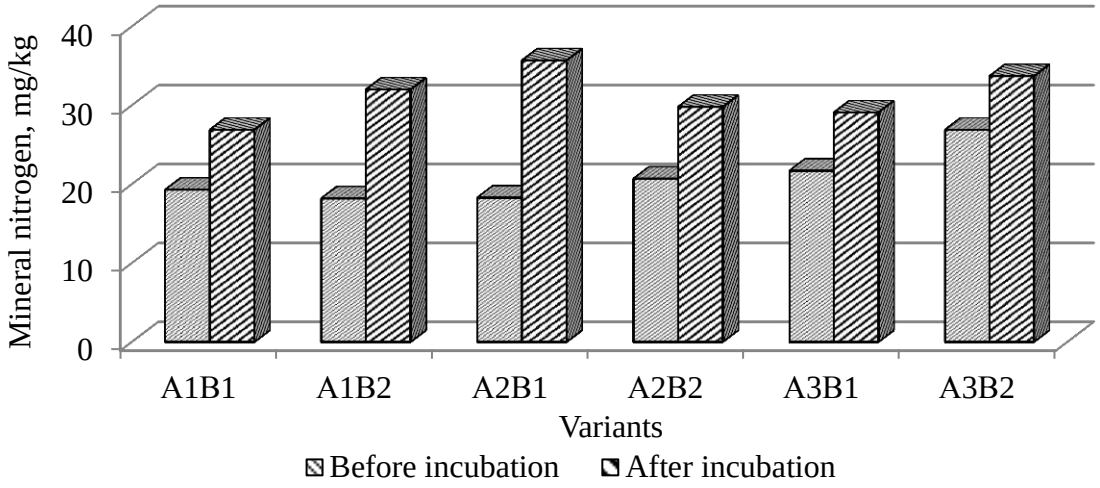


Figure 4. Impact of mobile phosphorus (A) and potassium (B) on the content of mineral nitrogen in the soil, mg/kg

Thus, prior to incubation mineral nitrogen content was 19.3 mg/kg of soil in variant A_1B_1, and after incubation 26.9 mg/kg of soil. A similar trend was also noted in other variants of the experiment. The most significant difference in the content of mineral nitrogen is marked in the variant A_2B_1 17.4 mg/kg of soil. The data indicate that under conditions favorable for the soil nitrification process a significant amount of mineral nitrogen can be accumulated. However, even those created favorable conditions for the accumulation of mineral nitrogen in the soil do not provide a sufficient amount of nitrogen for winter rye.

Conclusion

Activity of microorganisms in sod-podsolic the heavy loam soils the very low. The content of mineral nitrogen in the soil depends on the content of labile phosphorus and potassium, which suggests an ability to predict the future availability of nitrogen for winter rye.

On soils with average, elevated and high phosphorus content and low and medium potassium under optimal conditions an amount of mineral nitrogen sufficient for the growth and initial development is accumulated. The best conditions for nitrification are marked on variants A_2B_1 and A_3B_2, worst on A_1B_1 and A_3B_1.

For cultivation of winter rye on sod-podzolic soils of heavy texture with phosphorus level above average and potassium level low to medium, growing technology should provide nitrogen fertilizer that will provide the plant with necessary nitrogen.

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SOIL EROSION IN THE ČANČAR BROOK CATCHMENT (WESTERN SERBIA)

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Abstract

Soil erosion involves detachment and transport of soil particles from the upper parts of a slope and their deposition at its lower parts. Erosion causes soil degradation and reduces soil productivity. Soil eroded from the upland catchment causes depletion of fertile agricultural soil and the resulting sediment deposited at the river networks creates river morphological change and reservoir sedimentation problems. As regards the initiation of the wearing away of soil particles from the catchment slope, standard methods were used to quantify soil erosion. The aim of this study was to evaluate erosion factors in the catchment area of the Čančar brook, classified as a ravine. The annual erosion intensity is 113.23 m^3 km^{-2} of soil. Based on natural and anthropogenic factors, the Čančar brook catchment area belongs to erosion category IV, weak intensity, deep type, with the erosion coefficient of 0.40.

Keywords: *soil erosion, erosion intensity, sediment yield, soil, catchment, ravine*

Introduction

Land degradation and soil loss are global events. Human induced pressures on natural ecosystems are still in progress, along with conservation efforts (Hacisalihoğly et al., 2010). The main factor causing soil degradation worldwide is water erosion, which threatens 56% of the world's arable land (Oldeman et al., 1990).

Over 90% of the total land area in the Republic of Serbia suffers from different types and intensities of erosion (Djorović and Kadović, 1997). The erosion process can have both direct and indirect impacts, inducing permanent soil disappearance. The calculated value of the total annual sediment yield suggests that some 16.0 cm of soil are annually eroded off the 21,000 ha of land in Serbia (Spalević, 1997). In the Republic of Serbia (Central Serbia), there are 1.221 million ha of eroded soil, and 36,000 ha are in a steady state, now (Statistical Yearbook, 2008).

Erosion has mostly affected strongly sloping, deforested or cultivated shallow soils on slopes, formed on impermeable geological substrates, due to the effects of intense rainfall and fluctuating air temperatures (Spalević, 1997).

The tendency of air temperature to increase and of rainfall to decrease is quite evident in the region of Čačak (Šekularac, 2002). Climate change leads to degraded soil physical properties, increases soil erodibility and reduces the protective role of vegetation.

The above factors cause intensification of both surface and deep-cutting processes of erosion.

Given the above, the objectives of this study are quantitative assessment of soil erosion induced by a range of factors and estimation of sediment yield in one part of the catchment area of the Kamenica River (part of the Zapadna Morava catchment) i.e. its subbasin the Tinja, including its second order left-hand tributary the Čančar brook.

Materials and methods

The Čančar brook is located near Čačak (43^0 53' N; 20^0 21' E), Western Serbia, and belongs to the catchment of the Zapadna Morava river.

Figure 1. The Čančar brook catchment

Natural characteristics of the Čančar brook basin were studied using map data (hydrography, relief, geological substrate and soil), literature data (elements of climate: rainfall and air temperature) and data obtained through an immediate reconnaissance survey of the area (vegetation).

Maps of the studied area have the following scale: topographic map (1:25,000, Fig. 1; 1:50,000) by the Military Geographical Institute (1971), geological map (1:500,000) by the Institute of Soil Science (1966) and pedological map (1:50,000) by the Institute of Soil Science (1964).

Meteorological parameters for the catchment area were calculated using the method of interpolation of rainfall data (Republic Hydrometeorological Bureau, 1930-1961) by the rainfall gradient (Bonacci, 1984), and air temperature (Centre for Research in Agriculture, 1949-1995) calculations for any altitude (Dukić, 1984).

Erosion-induced soil losses can be predicted by various analytical models.

However, according to the experience of a number of researchers, the Erosion Potential Method – EPM (Gavrilović, 1972) is the most suitable on catchment level for watershed management purposes in this Region and is used in: Bosnia & Herzegovina, Bulgaria, Croatia, the Czech Republic, Italy, Iran, Montenegro, Macedonia, Serbia and Slovenia (Behzadfar et al., 2014; Spalevic et al., 2014; Kostadinov et al., 2014). This is why quantitative indicators of soil erosion in this research were calculated using the Erosion Potential Method - EPM.

The basic analytical equation for the calculation of erosion-induced soil losses, as developed by Gavrilović (1972), is as follows:

$$G_{yr\times sp^{/1}} = T \times H_{yr} \times \pi\sqrt{Z^3} \times R_u \qquad (1)$$

where:
$G_{yr\ sp}^{-1}$ – specific annual total erosion-induced sediment yield reaching the confluence, $m^3\ yr^{-1}\ km^{-2}$
T – temperature coefficient of the catchment
H_{yr} – amount of rainfall, mm
π – 3.14
Z – coefficient of erosion
R_u – coefficient of retention of soil in the catchment.

Results and discussion

The size, length, circumference and shape (perimeter) of a catchment area are among major catchment elements of importance for soil erosion. The Čančar brook catchment is 0.54 km^2 in area (F), 1.36 km in length (L), and 3.70 km in circumference (C).
The major physical and geographical elements of the Čančar brook catchment, relief characteristics, geological substrate features, soil type and soil utilisation method, are quantitative parameters or soil erosion in the catchment.

Table 1. The basic parameters of the Čančar brook catchment relief

Catchment Name: The Čančar brook	
The lowest point of the main watercourse and catchment (B), m	619
The highest point of the main watercourse (C), m	745
The highest point of the catchment (E), m	762
Average slope of the main watercourse in the catchment (I_a), %	8.3
Mean catchment altitude (A_m), m	700.74
Mean catchment altitudinal difference (D), m	81.74
Mean catchment slope (I_m), %	20.1
Coefficient of catchment relief erosion energy (E_r), $m/km^{1/2}$	53.16

Table 1 presents the Čančar brook relief which plays a primary role in the occurrence of soil erosion. The mean altitude (A_m) of the Čančar brook is 700.74 m and the mean altitudinal difference (D) is 81.74 m. The mean slope (I_m) is 20.1%. Relief of a region can also be determined by the coefficient of relief erosion energy (E_r), the value thereof for the Čančar brook catchment being 53.16 m $km^{-1/2}$. An increase in relief parameter values results in increasing intensity of soil erosion in the catchment.
Geological substrates contribute significantly to the erosion process within the Čančar brook catchment area (Table 2). Erosion resistance of geological substrates is directly related to water permeability. The geological substrate of the Čančar brook catchment is serpentine (100.00% of the total catchment area) and is with poor permeability. The water permeability coefficient of the serpentine geological substrate (S_1) is 1.00, suggesting non-resistance of the geological substrate to the erosion process (Table 2).

Table 2. Geological substrate of the Čančar brook catchment, coefficient of water permeability (S_1) and erosion resistance

Catchment name: The Čančar brook			
F_{ppr}-Poorly permeable rocks			
Serpentine		km^2	0.54
		%	100.00
Coefficient of geological substrate water permeability (S_1)		1.00	
Resistance of geological substrate to erosion		Non-resistant	

As an erosion agent, soil and its properties contribute, to a lesser or greater degree, to the erosion process. Due to the effect of pedogenetic factors, the soil type covering the Čančar brook catchment area is humus-siliceous soil on serpentine rock. It is classified as shallow soil. The profile of the humus-siliceous soil on serpentine is of A_h–C type. A strong degree of erodibility is found in the humus-siliceous soil on serpentine (Šekularac, 2000).

Table 3. The structure of the Čančar brook catchment according to type of land use and vegetative cover coefficient (S_2)

Catchment Name: Čančar brook			
F_f	Forests and coppice of good spacing	km^2	0.02
		%	3.70
F_g	Orchards	km^2	0.00
		%	0.00
	Meadows	km^2	0.07
		%	12.96
	Pastures and devastated forests and coppices	km^2	0.30
		%	55.56
$\sum f_g$		km^2	0.30
		%	68.52
F_b	Arable land	km^2	0.03
		%	5.56
	Infertile soil	km^2	0.12
		%	22.22
$\sum f_b$		km^2	0.15
		%	27.78
Vegetation cover coefficient (S_2)		0.84	

The most aggressive climate elements inducing and contributing to soil erosion include rainfall, air temperature, and soil temperature (indirectly, through air temperatures). This region has a temperate continental climate. The mean annual rainfall total (R) for the Čančar brook catchment is 799.1 mm, and the mean annual air temperature (T) is 8.1^0C. The data on rainfall reaching the catchment surface indicate an important role of rainfall as a climate element in soil erosion in the catchment area observed.

The contribution of the other soil erosion agents i.e. vegetation, both autochthonous and anthropogenic, and vegetative cover coefficient (S_2) is given in Table 3. The total area of land under forests and coppice of good spacing (F_f) in the Čančar brook catchment is 0.02 km^2 (3.70%), most of the land – 0.30 km^2 (55.56%) is under grass vegetation ($\sum F_g$), and 0.15 km^2 (27.78%) of land are under bare soil ($\sum f_b$). These forms of land-use facilitate the protection of the studied area against erosion (vegetative cover coefficient, $S_2 = 0.84$).

The devastating potential of the watercourse can be determined from the hydrographic and hydrologic traits of the region analysed. The traits pertaining to the family of the Čančar brook torrent (F_c) are as follows: F_c: F; IV; Z=0.40, meaning that the Čančar brook is a ravine

(F) classified as class IV of erosion category (a deep type of erosion) and having an erosion coefficient (Z) of 0.40 (weak erosion intensity).
The above traits of the erosion factors in the Čančar brook catchment result in sediment production and soil erosion of particular intensity.
The scale of erosion of the Čančar brook catchment is manifested through the mean annual erosion-induced sediment yield, W_{yr} of 321.80 m^3 yr^{-1}.
The mean annual volume of the total sediment yield (G_{yr}) reaching the Čančar brook confluence is 61.14 m^3 yr^{-1}, whereas the specific annual total erosion-induced sediment yield reaching the confluence with the Kamenica River ($G_{yr\ sp}^{-1}$) is 113.23 m^3 km^{-2} yr^{-1}. This finding regarding the weak erosion intensity is comparable to that on the low-intensity erosion of the Grliška River region (Eastern Serbia) of ($G_{yr\ sp}^{-1}$) 209.12 m^3 km^{-2} yr^{-1} (Stefanović et al., 2007). Using the method of EPM, in research of the Djuricka river basin (North of Montenegro), predicted that the soil losses were 645 m^3 km^{-2} per year (Spalevic et al., 2013). The said erosion intensity on the Čančar brook catchment is manifested through the relief erosion energy coefficient of 53.16 m $km^{-1/2}$, the erosion coefficient (Z) of 0.40, mean annual rainfall of 799.1 mm and average annual air temperature of 8.1^0C, with about 68.52% of land area under grass vegetation ($\sum F_g$), and the dominating humus-siliceous soil on serpentine rock.
The above data show that, in view of the annual sediment yield, about 0.03 ha of soil up to 20 cm depth are eroded off the Čančar brook catchment area i.e. about 0.05 t ha^{-1} of soil are lost annually. The amount of the eroded soil material can be categorised as class I (0-1 t ha^{-1} yr^{-1}) of permissible or tolerable erosion (Hacisalihoğly et al., 2010).

Conclusion

The Čančar brook is classified as a ravine. The value of Z coefficient of 0.40 indicates that the river basin belongs to destruction category IV. The strength of the erosion process is weak, and deep erosion dominates in the studied area.
These and the other soil erosion agents analysed in the catchment area have resulted in the mean annual erosion-induced sediment yield of 321.80 m^3 yr^{-1}, and erosion intensity of 113.23 m^3 km^{-2} yr^{-1}. The erosion observed in this region is of weak intensity, and the anthropogenic factor is the key agent in the process governing soil utilisation, soil conservation and protection from further erosion-induced degradation.

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THE CONTENT OF HEAVY METALS IN THE BULEVARD SOIL IN NOVI SAD, SERBIA

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Abstract

Urbisols are considered as all soils in urban areas on which formation men had immediate impact. They are formed by mixing natural soil layers with laid down material. Heavy metals in natural soils originate from parent material, while the origin of these elements in urbisols also depends of the laid down material. In addition to these main factors, air pollutants also have influence on the dynamics of heavy metals in urbisols. Probably the biggest influence of air pollutants can be seen in the soils along roads, where the origin of heavy metals can be attributed to car exhaust fumes. Metals which most often occur as contaminants of these soil are cadmium (Cd), lead (Pb), mercury (Hg), cobalt (Co), chromium (Cr) and others. In this paper the results of the total content of heavy metals (Pb, Ni, Cd, Co and Cr) in urbisols along the Boulevard of Car Lazar, one of the most frequented roads in Novi Sad, are presented. In topsoil the highest values were found for Pb, Ni and Cr, while the values of Cd and Co are not too different from the control sample. As a control sample it is used soil from the park. The total content of Pb, Ni and Cr in the soil along the Boulevard is on average three to four, in some places five times higher than in the control sample. Compared with the maximum permissible concentrations (MPC), the values in the tested samples were enlarged, but do not exceed MPC. Compared to the deeper layers, surface layers of the soil had a higher content of heavy metals.

Keywords:*urbisols, the total content ofheavy metals*

Introduction

Soil is a mixture of a number of components, including organic matter, mineral constituents, all kinds of soil-borne organisms, water, air and man-made elements (Faiz et al., 2009). Depending on the location, soil can also contain pollutants originating from industrial emissions, waste disposal sites, agriculture, urban centers, or from emissions generated by motor vehicles (Modrzewskaet al., 2014). In urban areas, soils are formed by mixing natural soil layers with laid down material various origin. Urbisols usually have one or more layers and thickness of at least 50 cm, and the layers are composed of different materials that are formed by mixing, compaction, addition of substrate, synthetic materials etc. (Zemlyanitsky, 1963; Craul, 1985). Because of that characteristics urbisols are very various. Soil profiles show abrupt changes from one layer to another depending upon the constructional history of the soil properties while in most natural profiles gradually grade from one layer to the next, lower one (Craul, 1985). Nešić et al. (2014) said that the most vulnerable urban soils are soils next to road and they are almost totally changing by constant human activities. During the year that includes the various works on infrastructure networks, water supply, sewerage and electricity lines, and also to the roads and paths. Except this, roadside topsoil is affected by traffic pollutant which reduces their quality even more. In the past decades the number of motor vehicles has increased dramatically which means constantly rising contamination of the soil environment with heavy metals (Modrzewska et al. 2014). Most of the heavy metals

originate from exhaust fumes, oil leaks from cars, the wearing out of tires and brake disks and from corrosion of metal parts of vehicles, claim Faiz et al. (2009), Duong and Lee (2011), and Liu et al. (2012). Heavy metals found in soil are also derived from the lithosphere, being released during soil formation processes and therefore constituting the natural background of their total concentration (Ubavić, Bogdanović, 1995; Manojlović and Singh, 2012). The solubility and therefore the bioavailability of heavy-metal ions are very widely, because many factors influence their concentration in soil solution. The most important factors affecting metal availability are soil pH, clay content and organic matter content (Takáč et al. 2009). The most common heavy metals released from vehicles on road are cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn), cobalt (Co) and Chromium (Cr) (Faiz et al. 2009).
The aim of this research was to determine concentrations of some heavy metals (Cd, Pb, Ni, Co and Cr) in roadside soil along Car Lazar Boulevard in Novi Sad, one of the busiest roads in town.

Materials and methods

The study was conducted in Novi Sad which is the second largest city in Serbia and the administrative seat of both, the province of Vojvodina and the South Bačka District. Since its founding, the population of the city has been constantly increasing. In the 1990s and 2000s, the city experienced significant population growth and today city has 341,625 inhabitants (Statistical Office of Republic of Serbia). Novi Sad is located in the southern part of the Pannonian Plain, on the border of the Bačka and Srem regions, on the banks of the Danube river, facing the northern slopes of Fruška Gora mountain. Area in which the trial is conducted is in geomorphologic point of view of the alluvial terrace of the Danube. Urbisols, in this area of the city, are formed in natural soil type fluvisol, which is hydromorphic soil and belong to class of under developed fluvial soils with soil profile (A) or G (A)-C. These soils are recent, river, sea and lake deposits with layers. Pedogenetic processes are poorly expressed due to youth of deposit or because of sedimentation prevails pedogenesis. Physical and chemical properties depend on the number of layers and their thickness, texture, origin and sequence. Some typical characteristics are rarely discussed and they are different from profile to profile (Živanov and Ivanisević, 1986, Nešić et al., 2014). By the construction of defensive levees leakage of the river is prevented and consequently the natural process of genesis of fluvisol is stopped.
Field studies were conducted in three representative locations, next to the busiest intersections. Sampling was performed at two depths of 0-30 cm and 30-60 cm, as this is the part of the bulk of the solum developing roots. At each location it was taken six samples, three in shallow and three in deeper layer, in a triangle around the tree. Soil from a nearby park was taken as a control site. Soil samples, 24 in total, were air-dried and manually sieved through a 2 mm sieve for chemical and texture analysis. The collected samples were analyzed at the Laboratory of Soil and Irrigation and the Laboratory for Agrochemistry at Faculty of Agriculture, University of Novi Sad, with contemporary, recognized methods used for this type of research.
Laboratory studies include the determination of texture according to International B-pipette method with preparation in sodium pyrophosphate; pH value in the suspension of soil with H_2O and KCl, and the measurement is carried out using a pH meter "inoLab"; content of humus, the method of Tjurin and concentration of heavy metals extraction from soil using nitric acid by AAS (Atomic Adsorption Spectrophotometer).
For verifying existence of statistically significant differences it is used LSD test. Statistical analysis was performed with Dunnet test at the significance level of 95%. In this way entered data allowed to compare locality - a layer – conc. of metal. All the localities were compared with a control site, and because of that this test was used. The null hypothesis was that there

was no significant statistical differences in the content of heavy metals in all localities, while the alternative hypothesis was that content of heavy metals in localities is larger than control site, M> Contr.

Results and discussion

The results are shown in the Tables 1, 2 and 3. Based on results of soil texture it can be said that the soil of examined localities belong to textural classes of loam, with small variations, texture and composition which ranges from coarse sand to clay loam. In all samples the largest share of the grain-size composition has a sand fraction. The content of the clay fraction of the studied sites in the surface layer ranges from 13.53 to 17.50%, while the deeper layers of soil values range is from 9.83 to 16.18%. At control site clay content is lower, and ranges are from 6.36 to 4.23%. Urbisols of examined localities have a higher clay content than natural fluvisols in a protected part of the Danube at Novi Sad, while the content of this fraction in the control site is not different than natural fluvisols. (Nesić et al. 2014)

Humus content is higher in the shallow layer of the tested sites and it ranges from 2.31 to 2.60%. This is understandable, because the soil surface is covered by grass, which enriched surface layer in organic matter. The values in the deeper layers are less, an average of 1%, and the lowest value were in the control site, 1.11%. According to Dugalić and Gajić (2012), surface layers can be classified into medium humus soil, while the deeper layers are light humus soil.

Of the soil parameters soil pH is one of the parameters that affect significantly the share of bioavailable forms of metals (Takáč et al. 2009). The pH of tested sites measured in H_2O ranges from 7.70 to 8.62 as according Dugalić and Gajić (2012), meaning that these soils belonging to poorly or very alkaline soils. This confirmed the results of measurements of pH and in the suspension with KCl, according to which these soils belong to the alkaline (Ubavić, Bogdanović, 1995). Compared to natural soils in this area, or fluvisol being examined by Pekeč (2010), it can be said that the values of pH are almost identical.

Tab. 1: Clay content, humus, pH values and Pb, Ni, Cd, Co and Cr total content in topsoil

	Depth	Clay content	Humus (%)	pH		Heavy metals content mg kg^{-1}				
				H_2O	KCl	Pb	Ni	Cd	Co	Cr
Locality 1	0-30	17.50	2.46	7.70	7.55	93.54	25.69	0.74	10.56	19.68
	30-60	16.17	1.41	7.94	7.77	51.75	24.87	0.73	10.02	16.79
Locality 2	0-30	13.88	2.44	8.62	7.65	35.71	19.17	0.67	8.46	14.02
	30-60	9.60	1.33	8.30	7.79	18.36	22.72	0.63	8.58	15.02
Locality 3	0-30	13.53	2.31	8.12	7.83	53.19	21.76	0.75	8.36	14.61
	30-60	9.73	1.86	8.15	7.92	39.59	18.60	0.68	7.90	12.77
Control site	0-30	4.23	2.60	7.91	7.23	14.42	20.86	0.41	9.31	17.90
	30-60	6.36	1.11	8.01	7.66	6.13	8.51	0.32	4.35	5.53
			Limited value			100	50	3	30	100

In the analyzed samples values of lead were within the range of 16.39 to 104.63 mg kg^{-1}, while the control soils have much lower Pb concentrations, from 2.96 to 14.53 mg kg^{-1}. The average values do not exceed the MPC of 100 mg kg^{-1}, but it is evident that the values in the Locality 1 are higher compared to other sites, particularly with shallow layer. The shallow layers have higher levels of lead than deeper. When we compare the results of lead along the Boulevard with average values for southern Bačka (24.4 mg kg^{-1}), which give Manojlović and Singh (2012) we can conclude that they are generally increased by two to three times.

Average values of concentrations of nickel in the research areas ranges from 18.60 to 25.69 mg kg^{-1} in localities along the boulevard, while the values on the control site are less. In the surface layer of the control site pollution value is 20.86 mg kg^{-1}, and in a layer of 30-60 cm is 8.51 mg kg^{-1}. The MPC of nickel in the soil is 50 mg kg^{-1}. The higher value in the shallow layer is probably caused by air pollution. According to Johansson et al. (2009) or Khan et al. (2011), both lead and nickel come from exhaust fumes. Helmreich et al. (2010) added that another source of nickel contamination of soils near roads is the wearing out of the road surface and use of paints containing lead.

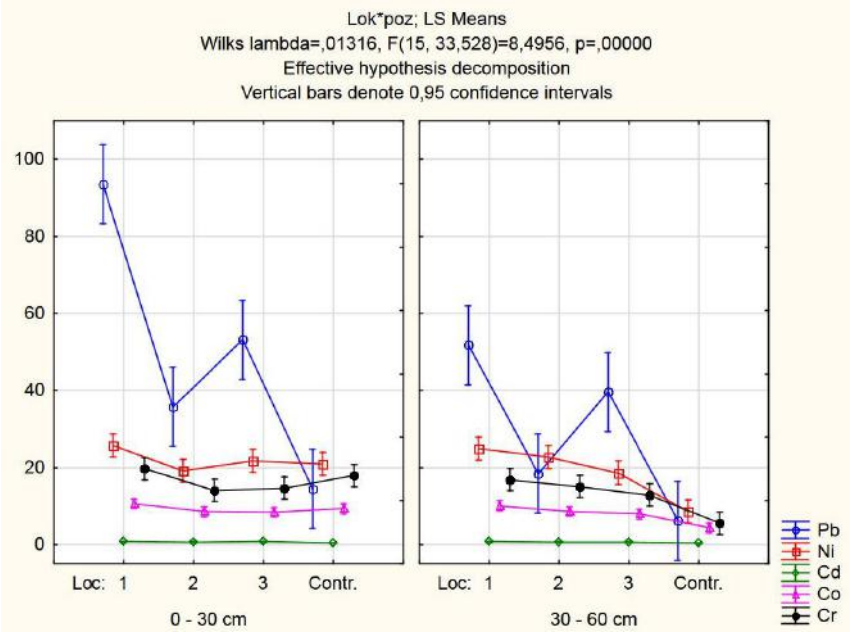

Fig. 1: Total content of Pb, Ni, Cd, Co and Cr in topsoil

Because of the very high toxicity of Cd for plants and to wild life, its MPC compared to other heavy metals are low (3 mg kg^{-1} of soil). On the tested sites content of cadmium is very low and does not exceed 1 mg kg^{-1} and so there is no danger of a negative effect of this element on the plant.

The values of cobalt content in the tested soil are below the MPC is 30 mg kg^{-1}. The highest content of this element has Locality 1 and it was 10.56 mg kg^{-1}. When we are comparing sites on the Boulevard in with the control site, it can be seen that there are no major differences.

The maximum level of chromium in the soil is the same like for lead is 100 mg kg^{-1}. In analyzed samples chromium values ranged from 5.53 to 19.68 mg kg^{-1}. When comparing the concentration of the surface and deeper layers it cannot be seen significant differences.

Contents of Co at all sites was at a low level because the coefficient of variation was 23.67%. The situation is similar with Cd where the average deviation from the mean value is 26.32%. The coefficient of variation for Ni amounts to 27.24%, and 31,15% for Co, which also indicates a lower average deviation from the mean value. These changes do not indicate greater variation in the amount of these heavy metals in the tested sites and control. Higher deviation of measured values of lead was observed at all sites. Even 70.58% is average standard deviation from the mean measured values. Minimum lead content was measured in a control sample and it was 2.96 mg kg^{-1} while maximum is measured in Locality 1 and it is showed in Table 2.

Tab. 2: Descriptive Statistics of measured samples

Variable	Descriptive Statistics						
	Valid N	Mean	Median	Minimum	Maximum	Std.Dev.	Coef.Var.
Pb	24	39.09	35.91	2.96	104.63	27.59	70.58
Ni	24	20.28	21.62	5.99	26.76	5.523	27.24
Cd	24	0.62	0.68	0.21	0.81	0.16	26.32
Co	24	8.44	8.85	2.74	10.86	1.99	23.67
Cr	24	14.54	15.69	4.06	21.56	4.53	31.15

Considering that the values of Ni, Cr, Co, Cd are far below the MPC, statistical analysis was not performed for these elements. Statistical analysis was performed with Dunnet test at the significance level of 95% for Pb. Some obtained data content of Pb in soil, adjacented to the maximum MPC. At the control site, between the deeper and shallow layers there are not statistical significance. Comparing pairs of site-layer there was a significant difference between contol and other localities, excluding locality 2 in a deeper layer. It can be said 95 % that the alternative hypothesis M> control it is comfimed.

Tab. 3: Dunnet test, M>Control

Dunnet test; variable Pb Probabilities for Post Hoc Tests (M>Control) Error: Between MS=70.266, df=16.000			
Locality 1	0-30 cm	0.000020	0.000020
	30-60 cm	0.000177	0.000036
Locality 2	0-30 cm	0.017382	0.001519
	30-60 cm	0.676677	0.183114
Locality 3	0-30 cm	0.000125	0.000031
	30-60 cm	0.005593	0.000498
Control site	0-30 cm	**14.425**	0.388614
	30-60 cm	0.995850	**6.1301**

Conclusion

Based on the research of texture, pH and humus content it can be said that urbisols on examined localities along Car Lazar Boulevard in Novi Sad belong to textural classes of loam, with small variations, texture and composition which ranges from coarse sand to clay loam. In all samples the largest share of the grain-size composition has sand fraction. As the consequence of the high content of sand fraction urbisols of this area does not have high capacity for cation adsorption. Humus content is at medium level of availability in the shallow layers, and at the low availability in the deeper layers. The pH value of tested samples is classified in the low to very alkaline. The total content of heavy metals is below ranges of MPC, but it can be seen increased content of lead and nickel compared to the control site. Because of that it can be said that the traffic flow had an effect on the content of heavy metals in roadside soils, which indicates increased risk of contamination of the soil with heavy metals in future.

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PRECIPITATION IN VOJVODINA PROVINCE AND THEIR EFFECTS ON CROP PRODUCTION

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Abstract

Serbian crop production is concentrated in the northern plains of Vojvodina region, inter alia due to the favorable agro climatic conditions. The climate is continental with Danube precipitation regime, with the most rainfall occurring in early summer and the least in the beginning of spring and in the mid-autumn. The precipitation recorded for the past thirty years (1985-2014) at the Bački Petrovac meteorological station had the average annual value of 616 mm (248-973), with 363 mm (103-636) falling in the crop vegetation period. On the thirty-year average, sum of precipitation increased 4.5 mm per hydrological year; respectively 3.4 mm in crop vegetation, 1.0 mm in winter and 0.4 mm in July-August period. However, the eight years with the most extremely high or low precipitation were within the 2000-2014 period, causing frequent fluctuations in crop yields. Average yields were in the intervals of 2.9-8.8 t ha^{-1} for maize, 1.2-3.6 soybean, 1.5-3.0 sunflower and 24.7-55.6 t ha^{-1} for sugar beet. The yield variation may be due to the precipitation variation in July-August, when the crops have the highest water demands. Their average monthly water requirement is about 100 mm; however, 83 and 90% (July and August, respectively) of the analyzed thirty years were characterized by the lower sum of the precipitation. Only five and two years were with precipitation in July and August higher than 125 mm. The number of tropical days (maximum daily temperature > 30^{o}C) also increased during the analyzed thirty-year period (0.4 per year, on average) affecting crop production.

Key words: *weather conditions, precipitation, drought, crop production*

Introduction

The agricultural plant production is influenced by numerous factors; soil and climatic conditions, the grown cultivar or hybrid, agro technical procedures, irrigation, pests etc. Out of the listed factors, the climatic conditions are under the least influence of the producers. The precipitation and the distribution of the precipitation during the hydrological year affect soil water balance and plant nutrition processes (Dragović et al., 2004). The lack of precipitation is related to droughts of the various intensity and duration which can cause yield losses (Dragović et al., 2005). Therefore, the monitoring and the analysis of the precipitation are of particular importance for crop production. Frequent variations in rainfall amounts are recorded in Vojvodina province known as our most important agricultural region. Rainfall values in certain smaller geographic areas of Vojvodina province can represent average annual rainfall of its extended area.

Therefore, the aim of this study was to analyze the influence of rainfall on the yield of the four most common crops in 30-year period.

Materials and methods

The meteorological data used for this analysis are collected from the archive of Bački Petrovac climatological station and include a thirty year period (1985-2014). The measuring point is placed in the central part of the Vojvodina region (N 45º 20´E 19º 40´, 82 m elevation). The sum, distribution and trends of precipitation were analyzed, with special reference to summer period when the droughts occur more often. Official data of the Statistical Office of the Republic of Serbia were used for the analysis of maize, soybean, sunflower and sugar beet yields.

Results and discussion

The analysis of the sum of precipitation noted at the Bački Petrovac measuring point in the thirty year period (1985-2014) showed the average value of 615.5 mm (Tab. 1). However, the interval of variation for the parameter was considerably high (385.8 – 1045.5 mm). Winter precipitation (X-III months) were on average 253.3, and those recorded for crop vegetation period (IV-IX) 362.2 mm. Both parameters were characterized by high intervals of variation.

Table 1. Mean annual, seasonal and monthly sum of precipitation (mm); Bački Petrovac, 1984/1985-2013/2014 period

Month	Winter precipitation		Month	Vegetation precipitation	
	Average	Variation		Average	Variation
X	52.4	3.0 – 2005 154.9 – 1992	IV	47.2	0.0 – 2007 111.0 – 2004
XI	48.1	1.5 – 2011 136.4 – 2004	V	66.6	10.2 – 1993 178.5 – 2014
XII	44.3	1.5 – 2013 121.6 – 1999	VI	80.1	18.7 – 2000 191.1 – 2010
I	37.4	2.8 – 1989 96.8 – 1987	VII	67.1	12.6 – 1988 173.6 – 1987
II	34.6	0.8 – 1998 72.6 – 1999	VIII	49.5	0.0 – 1992 142.1 – 2006
III	36.5	1.8 – 1992 93.5 – 2001	IX	51.7	3.1 – 1986 159.0 – 2001
Sum	253.3	73.4 – 1988/89 409.4 – 2009/10	Sum	362.2	103.0 – 2000 636.1 – 2010
Mean annual sum: 615.5 mm, minimum 385.8 (2011/2012), maximum 1045.5 (2009/2010)					

Maximum and minimum sum of precipitation for the thirty year period were noted for two consecutive hydrological years (2009/10, 2011/12), implying the unpredictability and inconstancy of the sum of precipitation. Considerably high deviations from the long-term averages are a characteristic of the precipitation in Vojvodina region (Spasova et al., 1999; Maksimović et al., 2013).

Table 2. Five hydrological years with the lowest annual sum of precipitation (mm); Bački Petrovac, 1984/1985-2013/2014 period

Hydrological year	Month												Annual	Veg. season	Winter period
	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX			
2008/2009	45	7	29	37	39	33	5	67	90	22	18	4	396	206	190
2011/2012	30	2	49	41	58	3	68	40	62	44	4	13	414	231	183
1999/2000	29	108	122	13	9	36	25	25	19	20	7	8	421	104	317
1989/1990	36	40	11	6	35	29	39	25	129	28	9	46	433	276	157
2002/2003	85	26	40	47	23	4	15	43	39	53	36	44	455	230	225
Average	45	37	50	29	33	21	30	40	68	33	15	23	424	210	214
Min	29	2	11	6	9	3	5	25	19	20	4	4			
Max	85	108	122	47	58	36	68	67	129	53	36	46			

Out of five the driest years, four are noted after the year of 2000 (Tab. 2). Since winter and early spring droughts are becoming more common, even the deeper roots cannot provide plants with water (Kresović, 2003).

Table 3. Five hydrological years with the highest annual sum of precipitation (mm); Bački Petrovac, 1984/1985-2013/2014 period

Hydrological year	Month												Annual	Veg. season	Winter period
	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX			
2009/2010	72	65	89	74	72	38	47	82	191	98	114	104	1046	636	410
2004/2005	83	136	38	27	41	39	30	39	120	161	137	66	917	553	364
2000/2001	8	28	52	42	25	94	110	81	174	30	16	159	819	570	249
1997/1998	92	42	79	67	1	23	41	98	111	75	84	78	791	487	304
2003/2004	121	26	15	53	46	19	112	136	106	45	51	38	768	488	280
Average	75	59	55	53	37	43	68	87	140	82	80	89	868	547	321
Min	8	26	15	27	1	19	30	39	106	30	16	38			
Max	121	136	89	74	72	94	112	136	191	161	137	159			

In addition, four of the five hydrological years with the highest sums of precipitation were within the 2001-14 period (Tab. 3), implying a high oscillation in the sum of precipitation (Fig. 1). Extreme years are becoming more often, either dry or rainy. Slight increasing trend was noted for sum of precipitation in hydrological years (4.5 mm per year), vegetation and winter period (3.4 and 1.0 mm per year, respectively). For July-August period this increase is not significant (0.4 mm per year). The results are not in accordance to the reports of Lalić et al. (2011), who used climatic models and scenarios to predict the decrease of annual sum of precipitation in the region of Vojvodina. Spasov and Spasova (2001) analyzed annual and seasonal rainfall time series in Serbia, in the period 1971-2000, and reported a decreasing trend for central Serbia, not only in annual, but, also in average rainfall during the growing season whereas a decreasing trend in rainfall in Vojvodina province over a growing season was less pronounced and no decrease in rainfall on an annual basis was observed.

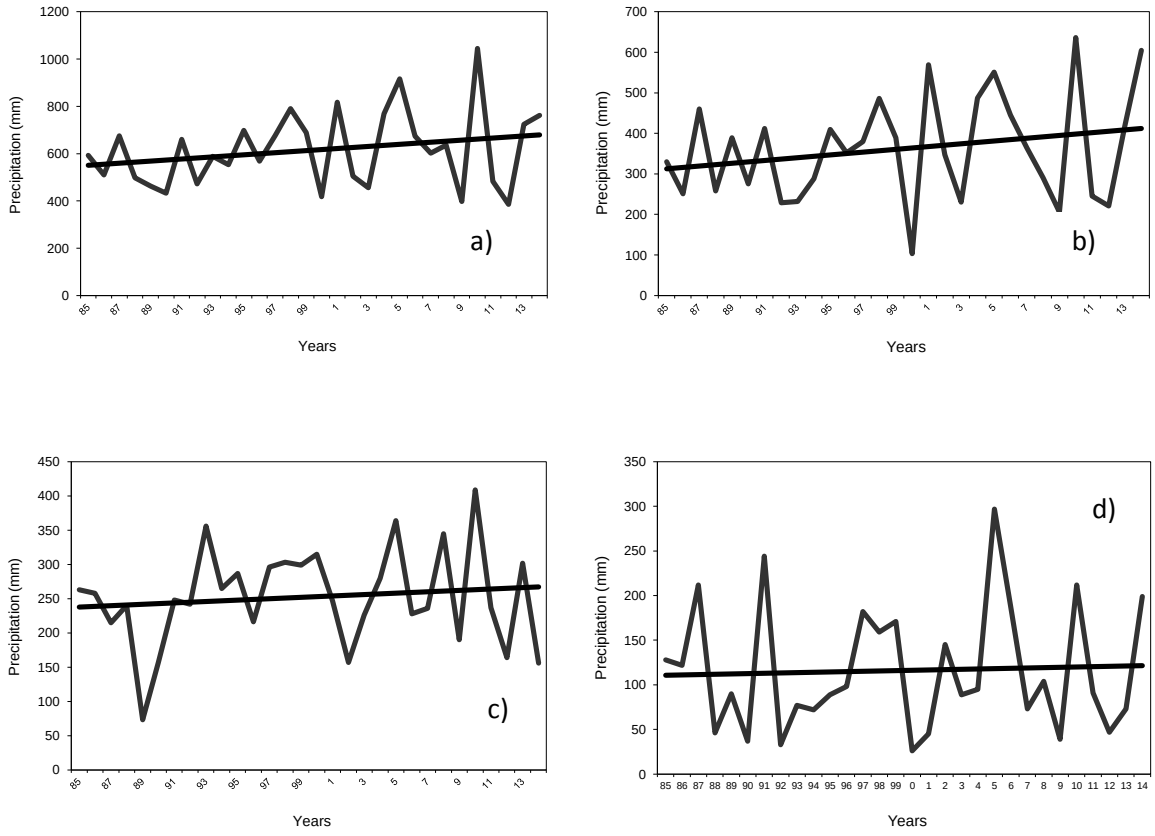

Fig. 1 Distribution of precipitation in Vojvodina (Bački Petrovac) – a) hydrological years; b) vegetation (IV-IX); c) winter period; d) July-August

Precipitation during vegetation is of particular importance for crop production, especially in July and August when most crops reach the phase of yield formation. Most crops require about 100 mm of woter in this period; therefore the value was used as a borderline between dry and rainy years. For the observed thirty year period, 90% of years were with dry August and 83% with dry July. Only five were rainy (>125 mm) in July and two years and August (Tab. 4).

Table 4. Dry and rainy years (%), based on sum of precipitation in July-August period; Bački Petrovac, 1985-2014

Rank	July		August		
(mm)	Number of years	%	Number of years	%	Category
0-25	5	17	8	27	Extremely dry
26-50	10	33	9	30	Very dry
51-75	7	23	6	20	Dry
75-100	3	10	4	13	Moderately dry
	25	83	27	90	Total dry
101-125	0	0	1	3	Moderately rainy
>126	5	17	2	7	Rainy
	5	17	3	10	Total rainy
Total	30	100	30	100	

Crop yields varied with the variation in sum and distribution of precipitation. The yields of four crops that are among the major in Vojvodina (maize, soybean, sunflower, sugar beet) and that are officially reported (Statistical Office of the Republic of Serbia) are given in Table 5; for five the driest and five the wettest years.

Table 5. Crop yields in Vojvodina region (t ha^{-1}) for five the driest and five the wettest years of the 1985-2014 period (Stat. Yearb. Serb. 1986-2014)

Precipitation (mm)-The driest years	Maize	Soybean	Sunflower	Sugar beet
247.7-2000	2.94	1.22	1.52	24.71
391.1-2011	6.01	2.71	2.52	50.96
425.0-1988	4.56	1.61	2.28	36.82
455.4-2012	3.04	1.74	2.24	36.48
455.6-1986	6.99	2.33	2.56	41.72
Precipitation (mm)-The wettest years	Maize	Soybean	Sunflower	Sugar beet
972.8-2010	6.71	3.21	2.25	50.15
863.4-2004	5.88	2.72	2.34	46.76
834.6-2001	5.59	2.39	1.98	42.69
807.2-2014	8.79	3.58	2.97	55.60
788.9-1999	5.35	2.75	1.48	42.32

The distribution of the precipitation is important for crop production. Satisfactory soil moisture in important plant developmental stages provides good yields, at the level of the years with generally good rainfall. The importance of the distribution of the precipitation can be illustrated with maize example: 1986 was dry year (455.6 mm); however, maize yield was higher (6.99 t ha^{-1}) than in four of the five the wettest years. Similar was for soybean and sugar beet, while sunflower yields were better in dry years (in most cases), related to fewer pest occurrence.

In addition, the increase in the number of tropical days (maximum daily temperature > 30°C) was noted for the analyzed thirty years (Lalić et al., 2011; Maksimović et al., 2014).

Conclusion

The analysis of the precipitation in the last thirty years (1985-2014, Bački Petrovac, central Vojvodina) showed the increase in the years with water regime which is unfavorable for spring crops. This is due to insufficient or excess rainfall and unfavorable precipitation distribution during the vegetation. Therefore crop yields vary, with significant losses in particular cases, such as the case of insufficient rain in July and August.

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SOIL EROSION IN THE ORAHOVACKA RIJEKA WATERSHED, MONTENEGRO

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Abstract

Erosion caused by water is a natural process and in the studied river basin is generated primarily by rainfall and surface runoff. In defining potential measures to alleviate the environmental effects in certain areas it is important to understand this process and to detect the erosion prone areas. We used analytical model IntErO for the assessment of the soil erosion process, soil loss potential and runoff. Peak discharge from the studied Orahovacka Rijeka Watershed, Q_{max}, was calculated on 174 m^3s^{-1} (for the incidence of 100 years); 155 m^3s^{-1} (incidence: 50 years); 141 m^3s^{-1} (incidence: 25 years); 101 m^3s^{-1} (incidence: 10 years). The value of Z coefficient of 0.478 indicates that the river basin belongs to III destruction category (of five). The strength of the erosion process is medium, and according to the erosion type, it is surface erosion. The calculated real soil losses, G_{year}, were 2923 m³ per year, 256 m³ per square kilometer. This study, being a part of the long-term research in this Region, repeatedly has shown that IntErO model is a useful tool for calculation of sediment yield at the level of the river basin for this part of Southeast Europe. Further studies, in the wider Region where the studied watershed belongs, should be focused on the detailed analysis of the impact of the climate change on the soil erosion intensity and runoff, as well as on the trends of land use changes, closely following responses of soil erosion to the changed land use structure on soil erosion and sediment dynamics.

Keywords*: soil erosion, erosion potential, IntErO model, sediment yield, peak discharge*

Introduction

Soil degradation and desertification risk is a globally acknowledged issue with ecological, socioeconomic, cultural and political implications at both the regional and local scales. Those processes occur in both developed and emerging countries and affect arid, dry and even sub-humid areas (Helming *et al.,* 2011).

Soil degradation caused by erosion, together with rapid population increase, are ranked as the most important environmental problems in the world (Stoffel and Huggel, 2012; Ristic *et al.,* 2001) where the erosion is a key driver of land degradation heavily affecting sustainable land management in various environments worldwide (Ballesteros-Cánovas *et al.,* 2015; Stoffel *et al.,* 2013; Verheijen *et al.,* 2009). Every year, soil erosion leads to the loss of about ten million hectares of cropland, which reduces the limited amount of arable land available for food production, thereby contributing to malnourishment in millions of people (Pimentel, 2006). In addition, the accumulation of large volumes of sediment can cause severe sedimentation in reservoirs and channel beds, resulting in the loss of various functions in these hydraulic projects. Thus, it is important to determine the sediment yield rates in watersheds, which can provide a good basis to facilitate soil erosion control and river basin management (Zhao *et al.,* 2015).

Soil erosion is serious problem globally, but also in Europe (Yassoglou *et al.,* 1998). Structured information on soil erosion for the Western Balkan countries are missing in the reports for the European continent, but various research results have been recorded by the scientists Kostadinov and Ristic for Serbia (Lukic *et al.,* 2016; Kostadinov *et al.,* 2014, 2007; Ristic *et al.,* 2013), Spalevic for Montenegro (Spalevic *et al.,* 2015a; Spalevic *et al.,* 2015b; Spalevic *et al.,* 2015c; Spalevic *et al.,* 2015d; Spalevic *et al.,* 2015e; Spalevic *et al.,* 2015f; Spalevic *et al.,* 2015g; Spalevic *et al.,* 2015h; Spalevic *et al.,* 2015i; Spalevic *et al.,* 2015k), Blinkov for Macedonia and Albania (Blinkov, 2012; Blinkov and Kostadinov, 2010). Quantitative information on soil loss is needed for erosion risk assessment. The modelling of the erosion process has progressed rapidly, and a variety of models have been developed to predict both runoff and soil loss. The authors of this study used the computer - graphic models (Spalevic, 2011; Spalevic *et al.,* 1999a) for prediction of soil erosion intensity from the catchment area – IntErO model (Spalevic, 2011).

The objectives of the study were to compute the sediment yield for the studied Orahovacka Rijeka Watershed of the Polimlje River Basin on the north-eastern part of Montenegro; testing the possibility of application of the IntErO model in the conditions of the Western Balkans Watersheds.

Material and methods

The study was conducted in the area of the Orahovacka Rijeka Watershed in Montenegro (Figure 1).

Figure 1. Study area of the Orahovacka Rijeka Watershed, Montenegro

In terms of geomorphology and climate, it is part of the natural entity of the Polimlje Region (2188 km^2 in Montenegro), with the Lim River (the total length of 234 km, 123 km in Montenegro) as a main watercourse. The surface area of the Orahovacka Rijeka Watershed is 11.4 km^2 and the shortest distance between the fountainhead and the mouth, Lv, is 5.2 km. The average slope gradient in the river basin, Isr, is calculated on 32% what indicates that in the river basin prevailing very steep slopes. The average river basin altitude Hsr, is calculated on 857 m.

Fieldwork was undertaken to collect detailed information on the intensity and the forms of the soil erosion, the status of the plant cover, the type of land use, and the measures in place contributing to the reduction or alleviation of the erosion processes. Morphometric methods were used to determine the slope, the specific lengths, the exposition and form of the slopes, the depth of the erosion base, the density of the erosion rills, the degree of the rills, and other relevant parameters. Different forms: the shape of the slope, the depth of the erosion base and the density of erosion rills were determined (Draganic *et al.,* 2015).

Climatological data were received from the Meteorological Institute of Montenegro. Pedological survey was based on the research of the Agricultural institute from Podgorica led by Fustic and Djuretic (from 1964 to 1988), who analysed the physical and chemical properties of all soils in this Region, including those in the study area of the Orahovacka Rijeka Watershed, but also on the basis of Soil survey conducted by Spalevic (2011).
Various methods for erosion risk assessment are used in Europe. Development of erosion risk assessment methods, soil loss calculation and erosion intensity estimation has a long tradition (Blinkov, 2010) applying the three types of approaches: qualitative approach, quantitative approach, and model approach varying in their characteristics and applicability.
For the obtaining data on forecasts of peak discharge from the basin and the intensity of the soil erosion we used the program package Intensity of Erosion and Outflow - IntErO (Spalevic, 2011) that is an integrated, second-generation version of the program "Surface and Distance Measuring" (Spalevic, 1999a) and the program "River basins" (Spalevic, 2000). The Erosion Potential Method – EPM (Gavrilovic, 1972) is embedded in the algorithm of this computer-graphic method. The EPM and the IntErO model are currently in use in all the countries of Western Balkans (Barovic *et al.*, 2015; Barovic and Spalevic, 2015; Curovic *et al.*, 1999; Kostadinov *et al.*, 2014; Vujacic and Spalevic, 2015; Spalevic *et al.*, 2014a; Spalevic *et al.*, 2014b; Spalevic *et al.*, 2014c; Spalevic *et al.*, 2014d; Spalevic *et al.*, 2013a; Spalevic *et al.*, 2013b; Spalevic *et al.*, 2013c; Spalevic *et al.*, 2013d; Spalevic *et al.*, 2013e; Spalevic *et al.*, 1999b), but also used by some scientist teams from Brazil led by Pedro Veloso Gomes Batista (2015), Italy led by Alberto Tazioli (2009), Sadeghi, Behzadfar and the others from Iran (Sadeghi, 1993; Amiri, 2010; Behzadfar *et al.*, 2015; Behzadfar *et al.*, 2014a; Behzadfar *et al.*, 2014b, Barovic *et al.*, 2015), Saudi Arabia led by Ali M. Al-Turki (Al-Turki *et al.*,. 2015).

Results and discussion

The geological structure and soil characteristics of the area. Our analysis, extracting the geological data from the Geological map of Montenegro, shown that the poor water permeability rocks prevail (92%); and a part of the basin with medium permeable rocks is 8%. The coefficient of the region's permeability, S1, is calculated on 0.98. Based on the research of Fustic and Djuretic (2000); Spalevic (2011) and our own research, the most common soil types in the studied watershed are: Dystric Cambisols (96%) and Eutric Cambisols (4%).
There is a highly variable *climate* and human pressure on the land in the Orahovacka Rijeka Watershed. The climate is continental, with cold winters and warm, dry summers. The average annual precipitation, Hyear, is 873.7 mm; the temperature coefficient of the region, T, is calculated on 0.99; the amount of torrential rain, hb, on 157.6 mm.
Vegetation and land use. Good vegetation cover reduces overland flow velocity and increases infiltration by protecting the soil against rain drop impact and reducing the erosive capacity of the rain (Asfaha *et al.*, 2015) whereas deforestation leads to increased peak discharge. According to the available literature, using Google maps, including the records from the field half of the studied area is covered with forests (51%). The rest is under grass (41%), and minor part of the area is plough-land and ground without grass vegetation (9%). The coefficient of the river basin planning, Xa, is calculated on 0.48. The coefficient of the vegetation cover, S2, is calculated on 0.71.
Soil erosion and runoff characteristics. The dominant erosion form in this area is surface erosion. Problems with overgrazing are recorded also all over the studied area.
The input data for calculation of soil erosion intensity are presented at the (Table 1).

Table 1. Part of the IntErO report for Orahovacka Rijeka Watershed, Montenegro

Input data			
River basin area	F	11.4	km²
The length of the watershed	O	14.59	km
Natural length of the main watercourse	Lv	5.21	km
The distance between the fountainhead and mouth	Lm	4.07	km
The length of main watercourse with tributaries	ΣL	5.21	km
Basin length measured by a series of parallel lines	Lb	6.52	km
The area of the bigger river basin part	Fv	5.73	km²
The area of the smaller river basin part	Fm	5.67	km²
Altitude of the first contour line	h0	600	m
The lowest river basin elevation	Hmin	537	m
The highest river basin elevation	Hmax	1212	m
The volume of the torrent rain	hb	157.6	mm
Average annual air temperature	t0	8.9	°C
Average annual precipitation	Hyr	873.7	mm
Types of soil products and related types	Y	1.1	
Basin planning coefficient	Xa	0.48	
Equivalents of clearly exposed erosion process	φ	0.3	

The coefficient of the river basin form, A, is calculated on 0.55. Coefficient of the watershed development, m, is 0.44 and the average river basin width, B, is 1.75 km. (A)symmetry of the river basin, a, is calculated on 0.01 (the river basin is completely asymmetric). The value of G coefficient of 0.46 indicates there is very low density of the hydrographic network.
The height of the local erosion base of the river basin, Hleb, is 675 m. Coefficient of the erosion energy of the river basin's relief, Er, is calculated on 116.93. The value of Z coefficient of 0.478 indicates that the river basin belongs to III destruction category. The strength of the erosion process is medium, and according to the erosion type, it is surface erosion. We calculated the soil losses from the Orahovacka Rijeka catchment on 2923 m^3yr^{-1}, specific (per km^2) 256 m^3yr^{-1}. Peak discharge from the studied, Q_{max}, was calculated on 174 m^3s^{-1} (for the incidence of 100 years); 155 m^3s^{-1} (incidence: 50 years); 141 m^3s^{-1} (incidence: 25 years); 101 m^3s^{-1} (incidence: 10 years).

Conclusions

The study was conducted in the area of the Orahovacka Rijeka Basin, the left-hand tributary of the river Lim in Montenegro. We calculated the soil erosion intensity and runoff using the IntErO model. The value of Z coefficient of 0.478 indicates that the river basin belongs to III destruction category. The strength of the erosion process is medium, and according to the erosion type, it is surface erosion. We calculated the soil losses from the Orahovacka Rijeka catchment on 2923 m^3yr^{-1}, specific (per km^2) 256 m^3yr^{-1} and the peak discharge on 175 m^3s^{-1} (incidence of 100 years). This study further repeatedly confirmed the findings of Spalevic (2011) in possibility of implementing the EPM and the IntErO model in Montenegro and in all the Balkans (Blinkov, 2012; Kostadinov, 2007), but also wider, such as in Italy (Tazioli, 2009), Brazil (Batista, 2015, 2016), Iran (Draganic *et al.*, 2015a; Draganic *et al.*, 2015b; Barovic *et al.*, 2015; Behzadfar *et al.*, 2015; Amiri, 2010; Sadeghi 1993). That leads to the conclusion that the IntErO model may be a useful tool for researchers in calculation of sediment yield for the catchments which don’t have sediment sampling stations.

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COMBINED APPLICATION OF CROP ROTATION AND LOW RATES OF HERBICIDES FOR WEED CONTROL IN MAIZE

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Abstract

Three maize growing systems are nowadays most present in Serbia: continuous maize cropping (15%), two crop rotation (maize -winter wheat - 60% and maize - soybean - 15%) and three crop rotation (maize - winter wheat - soybean - 5%). Despite to economic reasons, extended rotations are more sustainable than short-terms rotations, especially those one with legume crops that support reduction of N inputs and increase maize grain yield. Crop rotations facilitate the rotation of herbicides with different mode of action and are more effective in terms of planed weed control.

The aim of the research was to test effectiveness of combined application of crop rotation - maize continuous cropping, maize-winter wheat rotation and maize-soybean-winter wheat rotation and herbicides that are applied in recommended and reduced rates on maize weed control. Number of weed species and weed individuals per species and fresh biomass of manually uprooting weeds, were estimated in maize. Weed estimation was done four and six to seven weeks after the application of herbicides.

Weed association was composed of eleven most distributed species in all cropping systems: *Datura stramonium*, *Solanum nigrum* and two species from genera *Chenopodium* and *Amaranthus* and perennials *Convolvulus arvensis* and *Sorghum halepense*. Number of weed individuals and weed biomass were significantly lowered with herbicide application in all cropping systems and half of the full dose appears to perform nearly as well as the full dose.

Key words: *weeds, maize, rotation, herbicides*

Introduction

System or program of measures for Integrated Weed Management is usually define by the specific situation and level of weed infestation in the production area. In this regard, the preventive measures are used to prevent the spread of weeds and the introduction of new species while direct measures suppressing weeds directly. Among cropping measures it is important to properly complete the crop rotation (Spasojević et al., 2012; Simić et al., 2015). Crop rotation is effective and basic measure within Integrated Weed Management System (IWMS), (Bastiaans, 2010). By rotation of row and narrow crops and systems of measures for their production, including application of herbicides with different mode of action, weed community has no opportunity to become adapted and stabile.

In Serbia, there are three major maize growing systems: continuous maize cropping (15%), two crop rotation (maize -winter wheat - 60% and maize - soybean - 15%) and three crop rotation (maize - winter wheat - soybean - 5%) (Videnovic et al., 2013). Extended rotations involving legume crops reduce N inputs, increase maize grain yield and are more sustainable than current short-terms rotations (Stranger and Lauer, 2008). On the other hand, herbicides in recent time have to fulfil many tests during registration process in order to become approved as more environmentally friendly. They need to be effective and selective and do not make serious changes in weed community balance and encourage some troublesome weed species such as alien or perennial weeds, or emphasize resistant biotypes. For preservation of their

efficacy on the long term, IWM is also necessary (Claerhout et al., 2015). Studies showed that *Echinochloa cruss-gally* plants are more susceptible to some post-em herbicides than plants from long-term monocropping. Previous studies also showed that IWM tools such as crop rotation, provided sufficient weed control without any significant differences in yield and greatly reduced maize reliance on herbicides (Simić et al., 2012; Vasileiadis et al., 2015). Crop rotation determinates weed community composition, influences abundance of individuals and even seed bank richness (Simić et al., 2014b). Even, in crop rotations, competition pressure of weeds is weaker than in maize continuous cropping and highly dependent on herbicide application (Simić et al., 2015).

The aim of the research was to test effectiveness of combined application of crop rotations and herbicides that are applied in recommended and reduced rates on maize weed suppression.

Material and Methods

A field experiment was conducted during six years, 2009-2014, as a split-plot trial on slightly calcareous chernozem on the experimental field of the Maize Research Institute Zemun Polje, Belgrade, Serbia. The basic treatment was a plant production system: maize continuous cropping (MC), maize-winter wheat rotation (MW) and maize-soybean-winter wheat rotation (MSW). The hybrid ZP606, wheat variety Takovčanka and soybean variety Lana were conventionally sown within optimal periods in all production systems. Conventional tillage was used in the trial. A total of 30 t ha^{-1} of manure was incorporated in autumn of 2008 and then every third year in maize monoculture and MSW. In autumn of 2008 and 2010, 20 t ha^{-1} of manure was incorporated in two-crop rotation variant and every second year after. Different herbicide rates represented treatments in sub-plots. After sowing and prior to maize emergence the combination of isoxaflutole and acetochlor (Merlin 750-WG+Trophy 768-EC) was applied in two rates: HR- recommended (105 g a.i. + 1536 g a.i.) and ½ HR - half of the recommended rate (52.5 g a.i. + 768 g a.i.), while herbicides were not applied in the control. Each treatment had four replications. In wheat and soybean usual combination of herbicides for broadleaf and grass weed control was applied in whole variant.

Weed estimation was done four and six to seven weeks after the application of herbicides (I and II estimation). Samples were drawn randomly by the one square meter. Number of weed species and weed individuals per species and fresh biomass of manually uprooting weeds were estimated in maize.

Meteorological conditions were similar for maize production in 2009, 2011 and 2013. In 2013, in the middle of growing season (Jun and July), there were a lower amount of precipitation than in same period in 2009 and 2011 year. 2012 was extremely dry and hot, with absolutely unfavourable conditions for maize production.

Results and Discussion

Weed association was composed of eleven most distributed species in all cropping systems. Between them, annual broadleaf species *Solanum nigrum, Datura stramonium* and two species from genera *Chenopodium* and *Amaranthus* were dominant (Tables 1, 2 and 3). Number of individuals of perennial species *S. halepense* was even higher after some years in maize continuous cropping and in untreated control variants while *Convolvulus arvensis* was present as almost uncontrolled species even in a three crop rotation. In MSW crop rotation species *Hibiscus trionum* and *Cirsium arvense* were not detected after one rotation cycle.

Results shows that, in avreage for all herbicide treatments and years, the number of weed individuals decreased from MC (53.8 plants per m^2), trough MW (28.7 plants per m^2) to the MSW production system (16.9 plants per m^2), whic makes three crop rotation very effective measure for maize weed control (Simić et al., 2015).

Table 1. Weed species and number of their individuals (plants m^{-2}) in maize continuous cropping (I evaluation)

Weed species	2009			2011			2013			Average
	C	1/2HR	HR	C	1/2HR	HR	C	1/2HR	HR	
CHEHY	32	3	1	20	9		10	4	1	8.9
DATST	10	5	1	19	2	1	8	2		5.3
CHEAL	10			17	3		4	1		3.9
ABUTE	5	3		3	5	1	4	3		2.7
SOLNI	2			55			25			9.1
AMARE	3			23	2		5			3.7
AMAHY				19			7			2.9
CONAR	2	10	4	8	6	6	19	7	6	7.6
SORHA				10	2	5	18	8	5	5.3
CIRAR	4	2	1	10			5			2.4
HIBTR	2	2		4	3	2	2	3		2.0
Total	73	26	7	195	35	16	108	29	13	53.8

Table 2. Weed species and number of their individuals (plants m^{-2}) in maize-winter wheat rotation (I evaluation)

Weed species	2009			2011			2013			Average
	C	1/2HR	HR	C	1/2HR	HR	C	1/2HR	HR	
CHEHY	7	1		8			7	2		2.8
DATST	12	5	1	13			9	2		4.7
CHEAL	11			7		1	7			2.9
ABUTE	5	2					2			1.0
SOLNI	1			15			17			3.7
AMARE	1						55			6.2
AMAHY	1						3			0.4
CONAR	8	6	12	3	1	1	4	1	2	4.2
SORHA		2	2	2			12			2.0
CIRAR	3	3								0.7
HIBTR							1			0.1
Total	57	22	15	49	1	2	123	5	2	28.7

Table 3. Weed species and number of their individuals (plants m^{-2}) in maize-soybean-winter wheat rotation (I evaluation)

Weed species	2009			2012			Average
	C	1/2HR	HR	C	1/2HR	HR	
CHEHY	11	1		11	4	1	3.1
DATST	9	5	2	8	1		2.8
CHEAL	9	1		12	2		2.7
ABUTE	4	1		4	2		1.2
SOLNI	4			15			2.1
AMARE	3	1		9			1.4
AMAHY				4			0.4
CONAR	6	5	5	1	3	1	2.3
SORHA	2	1	1	3	1		0.9
Total	63	19	8	83	13	2	16.9

Weed individuals of perennial species participated in total weed individuals' number with 28.44% in maize continuous cropping, 24.04% in MW rotation and 18.93% in three crop MSW rotation, which illustrate the weed control efficacy on each cropping system. Weed species richness decreased from untreated control to the HR treatments in all cropping systems and years, especially in MW and MSW production systems. After two cycles of double MW crop rotation, number of weed individuals was almost two times smaller comparing to maize continuous cropping, in the same years.

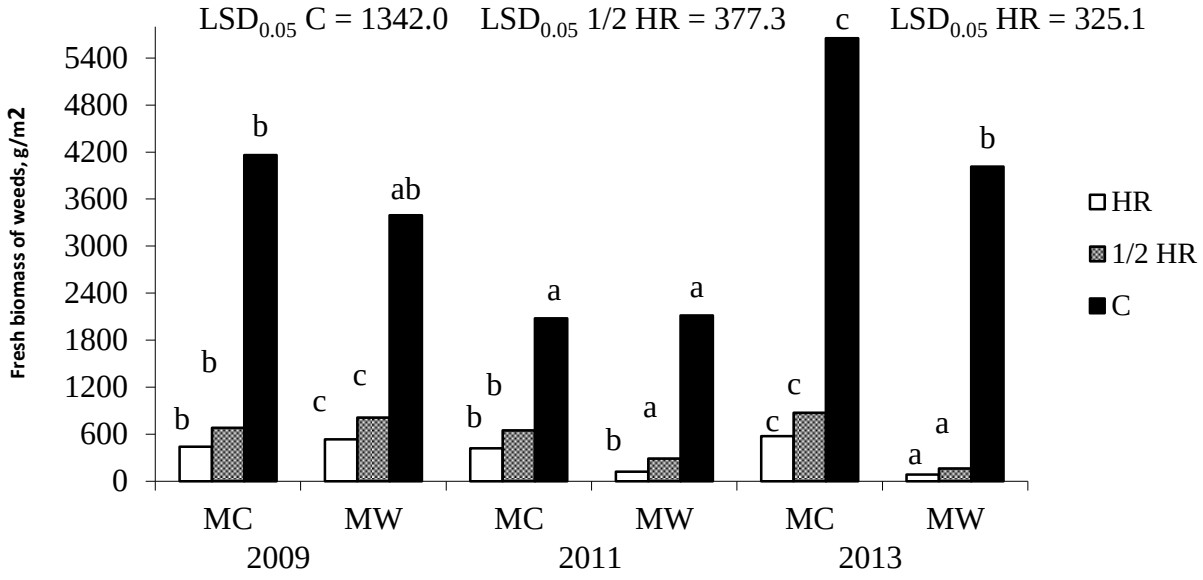

Figure 1. Biomass of weeds in maize continuous cropping (MC) and maize-winter wheat rotation (MW) independence of herbicide rates (II evaluation)

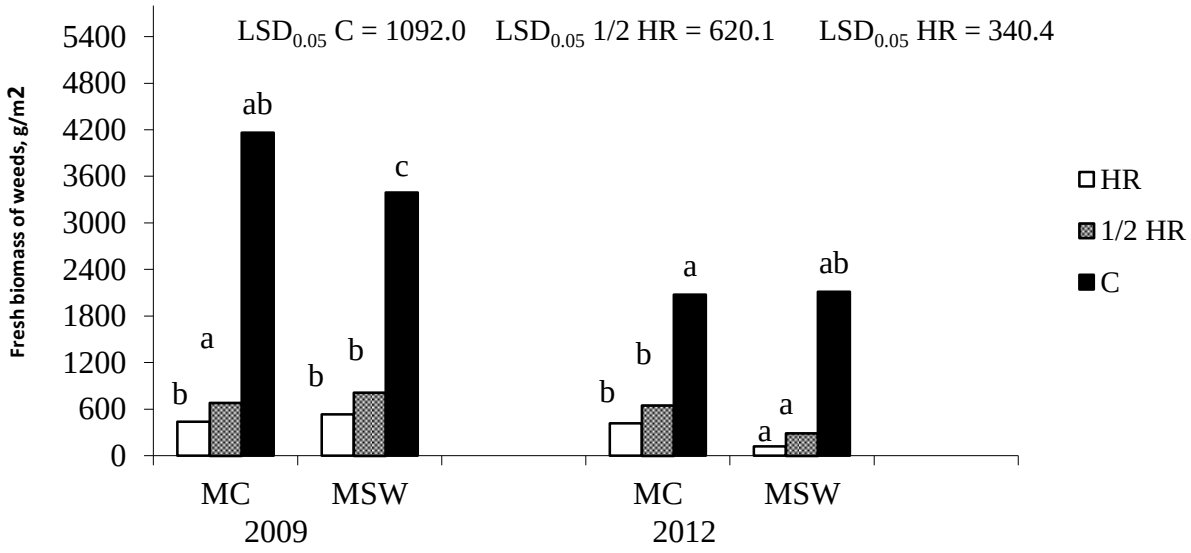

Figure 2. Biomass of weeds in maize continuous cropping (MC) and maize- soybean- winter wheat rotation (MSW) independence of herbicide rates (II evaluation)

Weed biomass was analysed in II evaluation, when present species and their individuals were fully developed and maize crop stand was almost completed (Figure 1 and 2). Weed individuals abundance and weed biomass were significantly lowered with herbicide application in cropping systems.

According to statistical analysis, differences in weed biomass between MC and MW, were higher after some rotation cycles (2013) than in the beginning of the experiment (2009). Similarly, the same regularity was observed between MC and MSW. Extremely dry and hot 2012 is exception and all measured values in this year were low. In most of the years, half of the full dose appears to perform nearly as well as the full dose.

Crop rotation and, due to it, the application of different herbicides, significantly contributes to control of annual, and especially of perennial weeds (Simic et al., 2014a). Weed species richness averaged over the rotation tended to decrease with herbicide application rate (Meziere et al., 20015). Wheat as a preceding crop, alongside with herbicides applied during the growing season as well as glyphosate-based herbicides applied to stubble fields after wheat harvest, greatly contribute to the reduction of weed infestation of maize as the following crop (Spasojevic et al., 2014). Even in especially dry 2012, when weeds tended to compete for water more than crops, the wheat x herbicide rate showed a great efficacy. Soybean as a component of three crop rotation system and herbicides applied during it’s growing, did not show significant effect on weed biomass decreasing probably because of completion of only one cycle of rotation.

Conclusion

The weed infestation level of maize crop can be successfully reduced by combined application of herbicides and crop rotation, even the simplest one such is maize-winter wheat rotation. Winter wheat as a cereal crop, together with an herbicide used for its production, was very effective for weed control. According to presented results, reduced herbicide rates together with rotations that include cereals or legume crops can facilitate suppression of weeds. The crop rotation should be implemented in a longer period of time in order to achieve reductions in weed abundance and higher maize yields.

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INTERNET COMMUNICATIONS IN THE STATE ENTERPRISE FOR FOREST MANAGEMENT „SRBIJAŠUME“

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Abstract

The State Enterprise (SE) for Forest Management “Srbijašume“ Belgrade manages forests sustainably, i.e. in a manner and to an extent that permanently maintains and improves their productivity, biodiversity, regeneration capacity and vitality, which in the present and future ensures fulfillment of economic, ecological and social functions of forests.

The forest is the most complex ecosystem and plays a particularly important role in preserving the environment. Serbia is considered a medium-forested country. Total forest area in Serbia is 2,252,000 ha, out of which 1,194,000 ha (53%) are state-owned, and 1,058,387 ha (47%) are privately owned. SE “Srbijašume“ manages state forests and forestland on the surface of 893,417 ha and carries out professional activities in forests of private forest owners on the surface of 1,058,387.00 ha (condition as of 31st December, 2014).

Internet communications (in the form of a website and Facebook profile) have been introduced into everyday forestry practice in order to raise people's consciousness about the importance of nature, especially forests, for further development of society,

Social networks are interactive social media that provide means for users to create their own web pages, themes, groups, photo galleries and to make online friends. They were initiated in the first decade of the 21st century. The phenomenon of socialization on social networks is copied from real life, it is just that cyber socialization happens faster and easier than in real society.

SE “Srbijašume“ uses its Facebook profile with the aim of raising people's awareness of the need for sustainable use, restoration, care and protection of forests as an invaluable natural resource and a key element of the environment.

Keywords: *SE "Srbijašume", Internet communication, environmental protection*

Introduction

The global environmental situation is alarming; we are faced with warming, pollution of soil, air and water, as well as ozone layer depletion, so the imperative need is that everyone gets involved in solving environmental problems (Meffe, 2013). Man's ruthless relation to nature has led human civilization into danger of irreversible destruction of the natural environment and consequently to deterioration of people's health and threatening their existence (Katelyn, 2013). Mankind has come into this dangerous situation due to the tendency of limitless pursuit of material wealth (Republic of Serbia, 2007).

Forests greatly affect the reduction of environmental problems. They mitigate the effects of global warming. More precisely, forests contribute to mitigating climate change by storing carbon in forest trees, branches, leaves, roots and soil.

Forests as the most complex ecosystems have a particularly important role in the preservation of the environment (Aleksić and Jančić, 2013). SE "Srbijašume's" strategic-environmental vision is obligatory for foresters to be responsible to the Serbian population. The Enterprise manages forests sustainably, i.e. in a manner and to an extent that permanently maintains and improves their productivity, biodiversity, regeneration capacity and vitality, which in the

present and future ensures fulfillment of economic, ecological and social functions of forests (http://www. srbijasume.rs/growingstock.htm, 2015).
New surface areas are regularly afforested and the existing ones are enriched. SE "Srbijašume" has its own 25 nurseries with a production of 14 - 16 million seedlings. Each year during the spring and autumn planting, it carries out the planting of 4 - 5 million new seedlings. New plantations are raised for the purposes of burned area rehabilitation and total forest cover increase in Serbia.
Serbia is considered a medium-forested country. Its forest coverage, when compared on the global scale, is close to the average of 30%, and it is significantly lower than the 46% (2000) in Europe.
Forest cover increase compared to the reference year of 1979 is 5.2%, which has certainly had a positive impact on the environmental conditions and quality in general. In relation to population, there is a forested area of 0.3 ha per capita (in Russia - 11.11 ha per capita, Norway 6.93 ha, Finland 5.91 ha, Bosnia and Herzegovina - 1.38 ha of and Croatia - 1.38 ha).
Total forest area in Serbia is 2,252,000 ha, out of which 1,194,000 ha (53%) are state-owned, and 1,058,387 ha (47%) are privately owned. SE “Srbijašume“ manages state forests and forestland on the surface of 893,417 ha and carries out professional activities in forests of private forest owners on the surface of 1,058,387.00 ha (condition as of 31st December, 2014).

Objective

The objective is to find the best ways for SE "Srbijašume" to communicate with businesses, potential polluters, NGOs and citizens, regardless of age, gender or education, since each person can affect forest conservation and environmental preservation by either doing or non-doing.

Materials and Methods

The paper is based on: European and American research on social networks; the study of the German NGO Magicspeed on electronic forms of information presentation on social networks; statistical data on the representation of social networks in Serbia; European experiences of citizen interest in Internet communication and citizen knowledge of the environment (Barry, 2012; Cavanagh, 2013; Magicspeed, 2014; Media Center Belgrade, 2013; World Journalism Observatory for EU, 2013).
Social networking service is an online service, platform or website that focuses on building and imaging of the social relations among people who share interests and/or activities. Although online social services are considered services of social networks in a broader sense, social network services usually refer to services focused on the individual, while the online community services are focused on social groups (Saxenian, 2012).
Social networks are interactive social media that provide means for users to create their own web pages, themes, groups, photo galleries and to make online friends (who do not necessarily have to be friends in real life), with a high degree of interconnectedness of all elements of the social network and care to independent determining of privacy settings by every individual. 'Social networks' is a term that came to life on the web in the first decade of this century and that had been preceded by forums and chat rooms (Cavanagh, 2013).
Early social networks on the web (the World Wide Web) were developed in the form of generalized online communities, such as: theGlobe.com 1994, GeoCities.com 1994 and Tripod.com 1995. Many of these early communities focused on bringing people together for the sake of mutual influence through chat rooms and encouraged users to share information and ideas through personal web pages. The characteristics of today's social networks are more advanced forms of social media, compared to forums or chat rooms. The new generation of

social networking sites flourished with the emergence of Friendster.com in 2002, soon becoming part of the prevailing direction of the Internet.
Facebook was launched with the renowned American university Harvard as a local student network, which, however, within short time experienced an expansion into all possible categories and social groups, from students to working people (Blank, 2009).
It is estimated that, today, there are over 400 active sites using a wide range of models of social networks. Most of the online communities can be divided into categories: Information, Professional, Educational and Entertainment. The phenomenon of socialization of this type is copied from the real society, it is just that cyber socialization happens faster and easier than in real society, but these cyber communication in the developed world represent the backbone for the establishment of international professional contacts both in business and at private level (Katelyn, 2013).
An important feature for programmers is the ability to independently develop their own applications for Facebook that promote their own services and facilities, thus redirecting part of the traffic to their own sites and building a reputation for themselves, but also for Facebook by making it more popular and useful.
Modern man is exposed to a large amount of information and data, of which only the most interesting and most important attract his attention. This was the basis for the study carried out by Magicspeed, the German NGO, on the types and content of information on social networks (Magicspeed, 2014). Part of the study was a survey asking the question “Which is the format of your favourite information on the social network: sound, text, multimedia?” The survey was conducted on a sample of 1,500 citizens of capital cities in three European countries: Germany, Austria and Poland in 2012 and 2013. Its results are presented in Figure 1, showing the greatest interest in the multimedia format in all three countries. These results are consistent with other studies that prove that people find visual learning of data to be most memorable (Cavanagh, 2013). The above survey shows the need of application of multimedia information to serve environmental protection. Facebook in Serbia is synonymous with social networking on the web (Media Center Belgrade, 2013). Statistical data for 2013 in Figure 2 clearly show that Facebook is the most popular in Serbia and that it is used by 37% of the population. SE “Srbijašume“ uses its Facebook profile with the aim of raising people's awareness of the need for sustainable use, restoration, care and protection of forests as an invaluable natural resource and a key element of the environment. (https://sr-rs.facebook.com/pages/srbijasume, 2015).

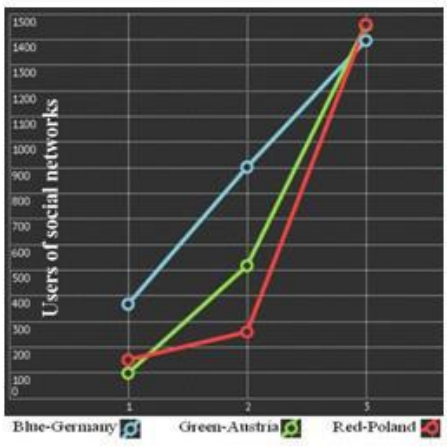

Figure 1. Overview of favourite information formats on social networks

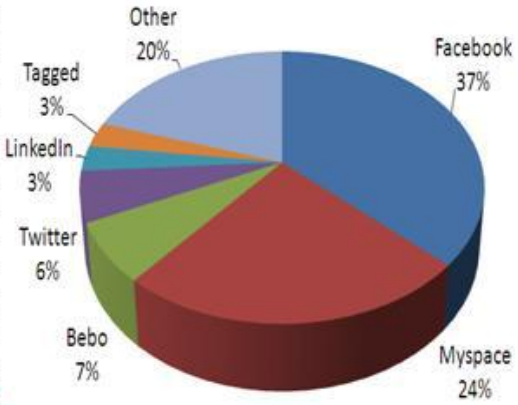

Figure 2. Representation of social networks in Serbia

Results and Discussion

Businesses around the world are increasingly oriented to Internet communications because representatives of their target groups' participation. This is a relatively new business communication channel, but there are great opportunities for expansion of new “green“ knowledge in order to raise awareness of the population to actively join the fight for nature conservation (World Bank, 2006).

“Green“ business has been a top priority for the EU for a decade now. Nine out of ten Europeans are aware that losses in biodiversity, i.e. reduction and potential disappearance of animal and plant species, natural habitats and ecosystems in Europe, are a serious problem (World Journalism Observatory for EU, 2013).

Europeans almost completely agree (93%) that it is important to halt losses in biodiversity since well-being and quality of life are based on nature and biodiversity (Kutz, 2013).

In order to raise people's consciousness about the importance of nature conservation, foresters of SE "Srbijašume" have introduced into their daily practice Internet communications in the form of a website and profile on the social networking site Facebook.

SE "Srbijašume" uses conventional and Internet business communication, as shown in Figure 3 (http://www.srbijasume.rs/basicdata.html, 2015). When it comes to Internet communications, first the website was applied.

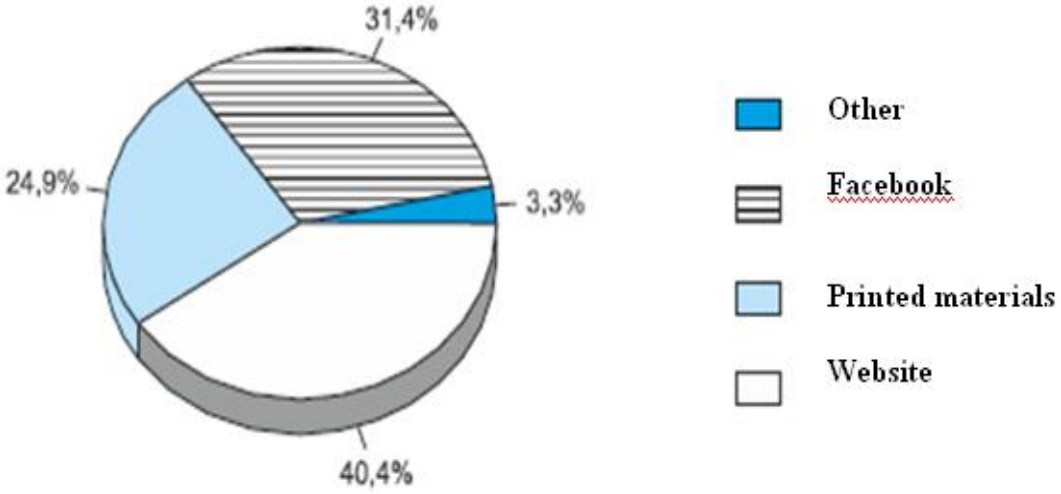

Figure 3. Types of Business Communication at SE “Srbijašume“

With the introduction of Facebook into communication, the number of website users has increased because young people who use Facebook every day have also accessed "Srbijašume's" profile, and from there have linked to the website and become regular users and our followers. With the introduction of multimedia content into Internet communications, the number of users has rapidly increased.

For efficient Internet communication it is necessary to fulfill three basic demands: updatedness, diversity and respect for the user. Data and information must be continuously changed, the materials placed must be from different areas of interest of the target groups, and every question or request of the user must be given adequate and quick response.

For the organization of in-field activities, the most effective communication is on Facebook, because large numbers of people can be quickly gathered for the purpose of extinguishing forest fires or collecting plastic bottles etc. Facebook is also effective for the placement of environmental education content.

Conclusion

Linking of social structures, economic entities and citizens is of crucial importance for the quality of the environment. In order to raise awareness about the importance of nature conservation for further socio-economic development, SE "Srbijašume" has introduced into their work practice, Internet networking to exchange information in the fight for a healthier and better environment. Internet communication has been chosen, because the Internet is a technological communication medium containing numerous services that make communication simple and accessible from any place and at any time.

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INFLUENCE OF LIGNIN CONTENT ON HIGHER HEATING VALUE OF BURLEY TOBACCO STALKS FROM DIFFERENT PRODUCTION AREAS IN SERBIA

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Abstract

Tobacco stalks make up a significant proportion of the total biomass in agricultural production in Serbia. They have no economic value yet, but may represent a large potential as a biofuel with amount of 70 000 tons per year.

The aim of this study is correlation between lignin content and higher heating value (HHV) of Burley tobacco stalks from two different production areas, Šabac and Čoka (Serbia). Lignin content determination was performed according the Zadražil and Brunnert method. The HHV of tobacco stalks as a function of lignin content (L, wt %) was calculated using the following equation:

HHV=0.0889 L + 16.8218

Statistical interpretation was performed SPSS v. 18. Statistical analysis showed that the lignin content and HHV of tobacco stalks are significantly different depending on the production areas. There was a higherly significant correlation between HHV and lignin content. Considering the high levels of HHV (19, 98 – 20, 51 MJ kg^{-1}) it is possible to use tobacco stalks as biofuels.

Key words: *higher heating value, lignin, tobacco stalks, Burley.*

Introduction

Reducing of fossil fuel reserve in the world encourages the much more implementation of renewable energy, which has great economic and ecological importance (Demirel, 2012). The use of renewable energy sources becomes significant energy issue in Serbia, and in particular will become important in the time of accession to the European Union. It is estimated that total of 12.5 million tons of biomass is produced in Serbia each year, of which 9 million tons in Vojvodina. The major part of agricultural waste in the Republic of Serbia makes corn stalks, wheat straw, and then sunflower remains (Malnar et al., 2014). A significant share of the crop residue makes also tobacco stalks (Kulić and Radojičić, 2011). According to the Statistical Office of Serbia (Statistical Yearbook of the Republic of Serbia, 2013), tobacco was planted on 6.200 hectares in the year 2013. After harvesting of large leaves tobacco on the fields remains a significant amount of crop residues, or an average of 22.000 stalks of tobacco per hectare. This means that about 76.000 tons of tobacco stalks remains in Serbia each year. These tobacco stalks have no economic value yet. It should be noted that this energy potential in our country is little used, because most farmers burn agricultural waste after harvesting, and the rest of biomass is plowed. Burning of crop residues in the fields adversely affects on land and on the ecosystem in generally, as it causes the release of large amounts of gases and from the other side, large amount of heat energy is lost in the atmosphere. This data indicates that unused tobacco stalks, which are classified into green tobacco waste (Radojičić et al., 2009), represent the potential that can be used in energy purposes.

It can be said that use tobacco stalks in energy purposes in the world is still unexplored in generally. In recent years researchers in Serbia are increasingly interested in the possibility of using these raw materials for energy purposes (Ećim-Đurić et al., 2014; Kulić and Radojičić,

2011; Malnar et al., 2014; Mijailovic et al., 2014; Radojičić et al., 2014a; Radojičić et al., 2014b). Based on these results the researchers agree that tobacco crop residues should be included in the energy balance and used it as biofuel.

The most important indicator of the possibility of using some organic raw materials as a fuel is its heating value, which depends on the type and chemical composition of biomass, as well as relative humidity. Thus, the tobacco stalks heating value, as well as for other biofuels, significantly depends on the content of organic compounds (Sheng and Azevedo, 2005; Yin, 2011). HHV of lignocellulosic fuels is a function of the quantity of lignin (Demirbas, 2001). Generally, HHV of lignocellulosic fuels increases with increasing lignin content in the fuel. The lignin HHV is from 23.26 to 25.58 MJ/kg. Pesevski et al. (2010), agree that high lignin content in tobacco stalks provides an opportunity for their use as biofuels.

Due to the increase in lignin content increases biomass heating value in the same time, defining the content of this substance (Demirbas, 2001) is quite important. The cell walls of the tobacco stalks can contain 40-45% of lignin, which is approximately the same amount as in softwood stalks. The only difference is that tobacco stalks have a significantly lower density of perennial trees wood.

Since the amounts and structure of lignin differ from one to the other types of biomass, it can be concluded that HHV vary from one to the other kinds of biomass. HHV can be obtained experimentally by determining in the bomb calorimeter. Experimental determination of HHV requires special equipment, and the element analysis data can be easily obtained with available laboratory equipment. Thus HHV fuels from renewable sources can be calculated using data on the content of lignin obtained by ultimate or proximate analysis. The aim of this study is determination of correlation between the lignin content and HHV from the organic material suitable for energy use, cultivated at different production areas.

Materials and methods

As a material for this research, Burley tobacco stalks from production area of Šabac and Čoka were used. Stalks were taken after tobacco leaves harvesting, at the beginning of October. Samples for laboratory analysis are prepared by grinding and homogenization. Lignin content was determined by Zadražil-in and Brunnert-in (Radojičić, 2011). For calculation of higher heating value, following formula was used (Demirbas, 2001):

$$HHV = 0{,}0889\ (L) + 16{,}8218 \quad (1)$$

Moisture was determined gravimetrically after drying the materials in an air circulation dryer, till constant weight, at 93±2°C (Radojičić, 2011).

Based on the moisture content, the results are expressed relative to the samples dry weight. All analyzes were performed in five replicates.

Data obtained from the experiments were analyzed and the results were expressed as mean ± SD. Statistics was performed using SPSS 18.0 software ANOVA. Pearson coefficient of correlation was used to determine correlation between variables (De Coster, 2004).

Results and discussion

Descriptive statistics indicators of pH and organic matter content in the soil from two production area, as well as the lignin content and higher heating value of Burley tobacco stalks are shown in Table 1.

Based on the data from Table 1, it is clear that the lignin content in Burley tobacco stalks is greater from Šabac production area than from Čoka. Given that the lignin content is in direct correlation with the HHV (Demirbas, 2001), the greater HHV was obtained for tobacco stalks from Šabac tobacco production area.

Table 1. Soil pH value, organic matter content in soil, lignin content and HHV of Burley tobacco stalks from Šabac and Čoka production area

	Origin	Average	SD
pH	Šabac^{SL}	6.1	.0707
	Čoka^{L}	5.6	.1225
Organ.matter %	Šabac	2.4	.1342
	Čoka	2.3	.0837
Lignin %	Šabac	41.56	.44816
	Čoka	35.48	.25456
HHV (MJ kg^{-1})	Šabac	20.51	.04099
	Čoka	19.98	.02490

Soil texture: L –loam; SL – sandy loam

Table 2. The significance of the difference in the observed parameters from two locations

	F	Significance
pH	62.500	.000
Organ.matter	2.880	.128
Lignin	695.773	.000
HHV	624.557	.000

Applying univariate analysis of variance (Table 2) it was established a significant difference in soil pH (** F = 62.5, p = 0.000), lignin content (F = 695.8 ** p = 0.000) and tobacco stalks HHV (F = 624.6 ** p = 0.002), but not in the organic matter in the soil between these two locations.

The relationship between soil pH, organic matter in soil, lignin content and HHV of Burley tobacco was investigated by using Pearson's coefficients of correlation (Table 3 and Table 4).

Table 3. Pearson's Correlation between soil pH, organic matter in soil, lignin content and HHV of Burley tobacco from Šabac production area

		pH	Organ.matter	Lignin	HHV
Šabac	pH	1	.264	.339	.345
	Organ.matter	.264	1	-.511	-.536
	Lignin	.339	-.511	1	.999**
	HHV	.345	-.536	.999**	1

**Correlation is significant at the 0.01

Table 4. Pearson's Correlation between soil pH, organic matter in soil, lignin content and HHV of Burley tobacco from Čoka production area

		pH	Organ.matter	Lignin	HHV
Čoka	pH	1	-.732	.000	-.082
	Organ.matter	-.732	1	-.423	-.336
	Lignin	.000	-.423	1	.994**
	HHV	-.082	-.336	.994**	1

**Correlation is significant at the 0.01

Based on the results shown in Tables 3 and 4, it can be concluded that there is a statistically very significant positive correlation between the lignin content and tobacco stalks HHV from selected production area (r = 0994 – 0999). Correlations among other examined parameters from both production areas are present, but they are not significant.

Organic matter content in the soil and the lignin content in stalks are negatively correlated, which is not statistically significant (r = -.423 – -.511). Since, there is no significant difference in organic matter content in the soil between locations (Table 2), it was concluded that organic matter did not affect on the lignin content, and therefore neither on HHV.

Based on the data in Table 3 and Table 4, it is evident that there is no statistically significant correlation between soil pH and lignin content in tobacco stalks, as well as between soil pH and HHV. However, applying the univariate analysis of variance (Table 2) it was found that there are significant differences in soil pH (F = 62.5 **) and lignin contents of tobacco stalks (F = 695.8 **) from selected production areas. Based on these results, it can be generally concluded that these two variables had an impact on tobacco stalks HHV.

Conclusion

Results obtained from this study indicate there is a significant difference between soils pH, lignin content and HHV of Burley tobacco stalks from different production areas. There is no difference between organic matter content in soil. The results also showed a statistically significant strong positive correlation between lignin content and HHV of tobacco stalks.
Considering the high levels of HHV (19, 98 – 20, 51 MJ kg-1) it is possible to use Burley tobacco stalks as biofuels.

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POTENTIAL CONTAMINATION ASSESSMENT OF THE WATER USED FOR SOIL IRRIGATION IN THE BASIN OF THE DRINA RIVER, SERBIA

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Abstract

The paper presents the results of analysis of the content of hazardous and harmful substances in the water used for irrigation, sampled during July, August and September in 2013, in the basin of the part of the Drina River, which flows through Serbia from Salaš Crnobarski to Bajina Bašta (Bušinsko polje). The study included 12 selected sites belonging to agricultural area under irrigation.

During the mentioned period it was determined the values of pH, ECw, TDS (total dissolved solids) and content of trace elements and heavy metals Cr, Ni, Pb, Cu, Zn, Cd, B, As, Fe, Hg in the water samples. The content of trace elements and heavy metals in the studied samples was generally below the maximum allowable concentration (MAC).

Based on the data obtained, it can be concluded that the water from the Drina basin is usable for irrigation of agricultural crops and soils, but with frequent quality checks during the summer months.

Keywords*: agricultural soil, hazardous and harmful substances, irrigation water*

Introduction

This paper presents the study of quality of irrigation water from the River Drina, based on the requirements of FAO (Food and Agriculture Organization of the United Nations) and U.S. Salinity Laboratory classification (Doneen and Westcot, 1988; U.S. Salinity Laboratory Staff, 1954), and designed for evaluation of usage of irrigation water. Irrigation is the artificial watering the soil in order to increase the moisture in the rhizosphere layer at a time when the amount of available soil moisture is insufficient to meet the optimum for the crops. Irrigation is a hydro-reclamation measure that aims to improve the physical properties of the soil by adding water to achieve optimum moisture during the growing season and thus achieve optimum yield. It may be applied during part of the growing season or during the whole growing season. For irrigation of cultivated plants on agricultural soil, water must have appropriate physical, chemical and biological properties, so it is very important to examine the quality of water for irrigation purposes. Intensification of irrigation depends primarily on the provision to the required amount of water of adequate quality.

Anthropogenic impacts and natural processes can affect the quality of surface waters and threaten their use as drinking water, and for use in industry, agriculture, and for other purposes (Carpenter et al., 1998; Jarvie et al., 1998; Simeonova et al., 2003).

The aim of this study is to assess the current water quality of the River Drina in order to be used for irrigation of agricultural soil near the streams and estimate the possible pollution risk. Pollution risks are mainly the direct consequence of the discharge of waste water from industrial plants, agricultural intensification or anthropogenic factors.

The major pollutants of surface water in the country are industrial enterprises, farms and settlements with sewage systems, without built facilities for waste water treatment, and such with acting, but technically outdated (Konstandinova et al., 2013).

Agricultural lands used for intensive agriculture and fertilized with nitrogen and phosphate, treated with pesticides, and manure from livestock farms are one of the major sources of diffuse pollution (mainly nitrogen, phosphorus and biodegradable organics).

Description of the study area

In geographical terms, the basin of Drina River in the flow through Republic of Serbia lies between 43°00' and 44°52' of north latitude and 19°15' and 19°20' of east longitude Drina River basin and locations of sampling are presented on Figure 1.

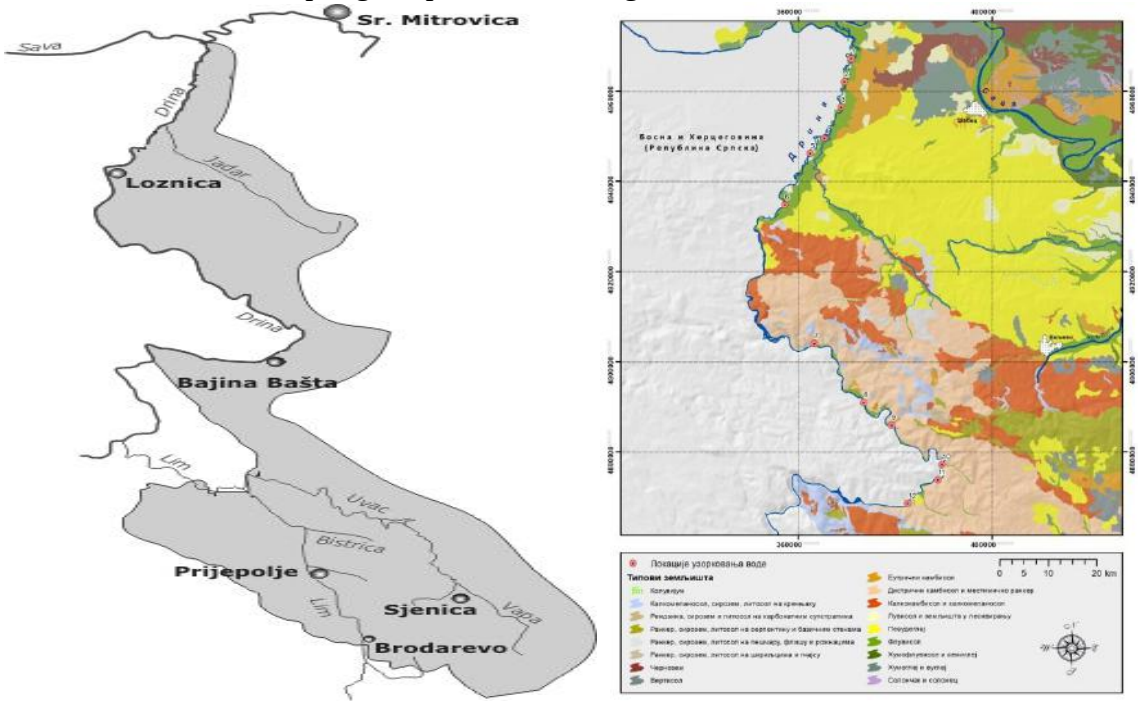

Figure 1. Location map of Drina River basin with selected sample sites.

The Drina is a 346 km long international river, and presents natural border between Bosnia and Herzegovina and Serbia. It is the longest tributary of the Sava River and the longest karst river in the Dinaric Alps which belongs to the Danube River watershed (Marković J., 1990 and Pivic et al., 2014).

River Drina basin covers the southwestern and western part of Serbia, the northern part of Montenegro and the eastern part of Bosnia and Herzegovina. Direction of its flow is from south to north and has a lot of tributaries. Water regime of the River Drina belongs to the typical modes of type snow-rain, with the primary peak of the water level in April and a secondary peak in December. Most of the stream is running through the mountains, while the entire upper stream is located in the high mountains of the Dinaric Alps, creating conditions that heavy precipitation and snows melting are leading to high flows. Decline of River Drina flow is very high, almost 2000 meters for less than 350 km of length, (spring is at about 2000 meters above sea level, while the confluence is on 80 meters above sea level). With an average flow of 395 m³ / s at the confluence, Drina is richest tributary of the River Sava.

Soils suitable for irrigation are primarily alluvial soils along the River Drina and meadow soils that are heavier texture from alluvium. Water physical properties of the soil along the Drina are very heterogeneous composition. Represented are applied gravel, sandy gravel, sandy, loamy and clay composition. All of these can be irrigated land varying amounts of water. The basic soil types of the studied area are Fluvisols, Eutric Cambisols and Distic

Cambisols (WRB, 2014. http://www.fao.org/3/a-i3794e.pdf, Accessed on 12/08/2015), presented by Figure 1.

Sampling and collection of water samples

Water samples were collected in three cycles of sampling, in July, August and September 2013, using 2000 ml plastic bottles from 12 (twelve) sampling points (Table 1), a total of 36 water samples were collected.. The sampling bottles for heavy metal determinations were pre-soaked overnight with 10% HCl, rinsed with distilled water and also rinsed using river water before sample collection. Sampling bottles for the determination of physicochemical parameters were cleaned and rinsed using distilled water only. Preservation of water samples was done by adding 2 drops of concentrated HNO_3 to each water sample before storage below 4°C until it was analyzed.

Table 1 Sampling points along the Drina River

Sampling point	Easting	Northing
1	7370890	4968150
2	7369520	4963040
3	7368840	4957430
4	7365400	4950450
5	7362500	4947150
6	7357260	4935790
7	7363290	4905030
8	7373510	4891940
9	7379220	4886950
10	7389660	4878210
11	7388832	4874792
12	7382590	4869580

Analytical methods

Parameters were determined by the following methods: temperature is determined *in situ* by a calibrated thermometer, pH-potentiometric (SRPS H.Z1.111:1987), electrical conductivity (ECw)- conductiometric (SRPS EN 27888:1993), TDS-gravimetric (Greenberg et al., 1998), The acid-available fraction of heavy metals and other toxic elements (As, B, Cd, Cr, Cu, Fe, Ni, Pb, Zn) was determined using EPA 200.7 methods, as well as an ICAP 6300 ICP optical emission spectrometer (ICP-OES). The concentration of Hg was determined by a flame atomic adsorption analyzer SensAA Dual (GBC Scientific Equipment Pty Ltd, Victoria, Australia).

Data processing methods

The experiment data were presented with mean of three tests with the presented summarized basic statistics of the dataset. Analysis of the interdependence of variables was carried out by calculating linear Pearson correlation coefficients. It has been assumed that the regression modeling of the potential usefulness of the selected variable (explanatory) to model another variable (explained variable) determines the absolute value of the high correlation coefficient between these two variables. The statistical analysis usually assumes that if the correlation coefficient is >0.9, a very strong linear dependence exists; 0.7-0.9-significant linear dependence; 0.4-0.7-moderate linear dependence; 0.2-0.4-distinct linear dependence, but low; <0.2-no linear dependence (Nelson and Sommers, 1996).

Results and discussion

The seasonal and annual averages of physicochemical characteristics are given in Table 2. The recorded water temperature in all three cycles of monitoring is in compliance with the prescribed values of national standards (Official Gazette of Republic Serbia, 23/94, 1994 and #50/12, 2012).

The pH value is a measure of alkalinity and acidity of the water. If the value is less than seven, the water or the aqueous solution is acidic, and if it is higher, then it is alkali. Plants for growth and development favor the slightly acidic solution, or the pH should be around 5.5. The pH is an important factor that determines the suitability of water for a variety of purposes, inter alia, for irrigation.

The tested samples showed pH values from neutral to slightly alkaline. In all series of monitoring the pH of the samples were in the range permitted by the classification USGA (Duncan et al., 2000), while in relation to the classification (Ayers and Wescot, 1994), is in the first series of monitoring.

Conductivity is a measure of the ability of an aqueous solution to carry an electric current. Increasing levels of conductivity and cat ions are the products of decomposition and mineralization of organic materials (Begum and Harikrishnarai, 2008). The aqueous salt solution and dissociated are broken down into positive and negative ions. Electrical conductivity in natural waters is generally with values less than usual. Measurement of the conductivity is performed at a specific temperature and it corresponds to the presence of dissolved salts. These are most commonly sodium chloride, and may be present, and sodium sulphate, calcium chloride, calcium sulfate, magnesium chloride, etc. Salts dissolved in the water influence on increase of the water conductivity values.

TDS are an important characteristic for determination of the quality of water for irrigation because it expresses the total concentration of soluble salts in water. Dissolved solids in water include all inorganic salts, silica and soluble organic matter. Pure water must be free from most suspended particles, which are responsible for turbidity. TDS was the highest in summer due to evaporation and reduced intake, which contributed to an increase in concentration, and had the minimum value in the rainy season, due to the increased entry of rain and a corresponding reduction in concentration at all locations.

Table 2. Average value of the water quality parameters of irrigation water, along with the standard limits by irrigation water US and FAO and by Republic of Serbia

Parameters	Mean±STDEV (n=36)	Standard limits		
		Duncan et al., 2000.	Ayers et al., 1994.	OfficialGazette, 1994/2012[#]
Temperature(^{o}C)	20.2±2.57			30
pH	8.17±0.17	6.0-9.0	6.5-8.4	
ECw 25˚C (dSm^{-1})	0.22±0.01		<0.7	<1.0[#]
TDS (mg·l^{-1})	282.88±126.36		0-2000	
As (mg·l^{-1})	0.0042±0.0025		0.1	0.05
B (mg·l^{-1})	0.014±0.006	2.0	0-2	1.0
Cd (mg·l^{-1})	bdl		0.01	0.01
Cr (mg·l^{-1})	0.0003±0.0002		0.1	0.5
Cu (mg·l^{-1})	0.0006±0.0002	0.2	0.2	0.1
Fe (mg·l^{-1})	0.015±0.0216	5.0	5.0	
Ni (mg·l^{-1})	0.0005±0.0003		0.2	0.1
Pb (mg·l^{-1})	0.0036±0.0019		5.0	0.1
Zn (mg·l^{-1})	0.0026±0.0031	2.0	2.0	1.0
Hg (mg·l^{-1})	bdl			0.001

*References (listed below in reference list); bdl-below detection limit

ə - in me/l = mill equivalent per liter (mg/l ÷ equivalent weight = me/l); in SI units, 1 me/l= 1 mill mol /liter adjusted for electron charge.

The contents of trace elements and heavy metals in the samples of water are generally below the MAC, maximum allowable concentration (Official Gazette of Republic Serbia, 23/94, 1994 and [#]50/2012, 2012).

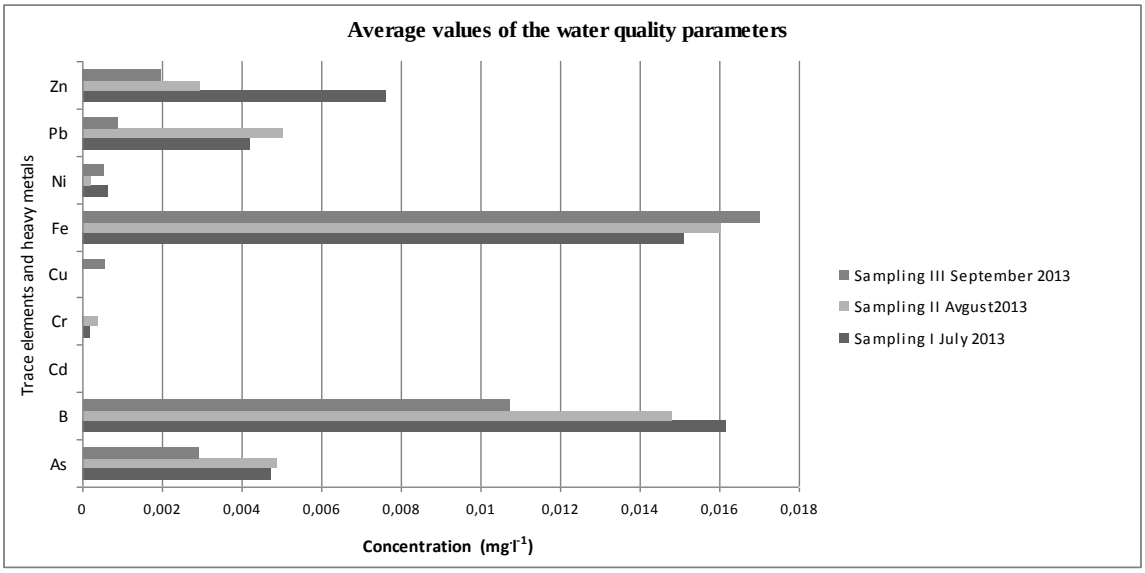

Figure 2. Average values of the water quality parameters of Drina River

The sources of metal pollution of natural water are: industrial and domestic wastewater, agricultural activities, mining, waste and intermediate products from power plants, factories for the production of non-ferrous metals, sludge from sewage treatment plants for municipal and industrial wastewater, as well as the processes of natural erosion and sedimentation (Aktar et al., 2010).

The diversity in the results and demonstrated dynamic variations, suggests impact of a number of various environmental factors on the pattern of metals distribution in the water. In this regard (Ozmen et al., 2004), reported that the concentration of metal ions strongly depend

on the biological processes, redox potential, ionic strength, pH, the activity of organic and inorganic chelators and the purification processes in water.

By analyzing of correlation parameters the conclusion is that the correlation between the samples and the characteristics of the water where the pH value has a significant negative correlation to all studied parameters except to the concentration of ECw and Cr (Atekwana et al., 2004; Gong et al., 2013). ECw values were negative correlated to all study parameters except to the concentration of TDS, B, Ni, Pb and Zn. TDS values show a positive correlation to the concentration of As, Pb and Zn, and a negative to the concentration of B, Cr, Cu, Fe and Ni in the samples of water.

The obtained results also implies on very strong linear correlation for As-Cr; As-Cu; B-Cu; Cu-Zn in the studied water samples, significant linear correlation for As-Fe; As-Zn; Cr-Fe; Cr-Zn; Fe-Zn, moderate linear dependence for B-Pb; B-Cr; B-Fe; Cr-Pb, and distinct linear dependence for ECw-As; ECw-Pb; TDS-As; As-Pb; while for the rest of observed parameters there is no linear dependence.

Conclusion

Results from obtained from water samples showed that the concentration of heavy metals in the water of the Drina River in most of the samples analyzed is within the MAC values. Variations of the heavy metal concentrations in the water are the result of a wide range of anthropogenic activities (primarily agriculture) in the study area throughout the year.

Based on the obtained and analyzed results of testing, the quality of water for irrigation from the Drina River can be used for irrigation of crops and soil with frequent quality checks during the summer months.

In detailed examination of usage of water for irrigation purposes in the area, it is necessary to extent the research with detailed study of hydrological parameters (determining the biological minimum of each watercourse), analysis of the amount of organic pollutants, study of the influence of temperature and oxygen concentration in the water on wildlife, etc.

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SEDIMENT QUALITY ASSESSMENT USING CULTIVATED PLANTS AS BIOINDICATORS

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Abstract

During seasonal maintenance of irrigation canals large quantities of sediment are dredged and need to be properly handled. The possibility of sediment use in agriculture has been intensively studied during last decades. Prior to its use it is necessary to assess the quality, detect present contaminants and characterize pollutants bioavailability to plants because contaminated sediment can pose a risk for crops. This work aimed to assess the quality of sediment (in the form of interstitial water) from canals Feketić, the Great Bačka canal –GBC and Stari Begej - special nature reserve “Carska bara” using cultivated plants as indicators. Biological effects were evaluated on physiological (germination) and morphological (root and shoot lengths and weights) responses of maize (*Zea mais L.*), radish (*Raphanus sativus L.*), cucumber (*Cucumis sativus L.*) and beans (*Phaseolus vulgaris L.*) according to a standard filter paper method (ISTA Regulations book, 2011). In sediment from Feketić and Stari Begej Ni and Cu were present in amounts exceeding maximal allowable concentrations according to regulations, while sediment from GBC was contaminated with Ni, Fe and Zn. In biotest, sediment from Feketić and GBC significantly inhibited germination of maize, radish and beans compared to the control and sediment from Stari Begej (F=19.78*, 260.80**, 80.63**, p<0.01/0.05, respectively), but highly significantly stimulated germination of cucumber (F=466.76**, p<0.01). Root and shoo lengths, fresh and dry weights of all test plants except cucumber were inhibited by sediment from Feketić and GBC, while sample from Stari Begej stimulated shoot lengths, fresh and dry weights of all plants compared to the control (p>0,05/0,01).

Keywords: *sediment, contamination, bioindicators, cultivated plants*

Introduction

During seasonal maintenance and revitalization of irrigation canals, sediment dredging is one of the most important activities (Savić et al., 2008). Large quantities of sediment which are dredged need to be properly handled and the use in agriculture is the most promising one, because they are rich in organic matter and nutrients. But also, sediment can be considered as a potential source of heavy metals, especially due to the fact that they are not permanently attached and immobilized (Rajić, 2010) and can be desorbed on the surface of particles and become available to organisms or exhibit ecotoxicological effects (Wasserman et al., 2013). Re-mobilization of metals from sediments is a major problem for the environment, because released metals affect the aquatic ecosystem as well as agricultural activities in the area (Middelkoop, 2000; Dennis et al., 2003) because they are usually heavily loaded with nutrients, hazardous or other undesirable substances. Far-reaching consequences are manifested in the irrigation canals, coastal region and the wider environment, in the form of degradation of the quality of irrigation water and useful disposal of sediments on arable land along the canals. The possibility of the use sediment on agricultural land reflects the need for a risk assessment on the presence of certain pollutants to plants (Bedell et al., 2006). Mainly, for sediment quality assessment and contamination detection, chemical methods are used. However, they solely are not sufficient because they do not provide information on the direct impact of pollutants and interactions on living organisms (Leitgib et al., 2007). Therefore,

they must be complemented with toxicological i.e. biological methods (Vaajasaari et al., 2002; Leitgib et al., 2007). Such integrated methodology gives information not only about content of contaminants, but also about bioavailability and allows complete risk assessment of contaminated sites (Leitgib et al., 2007). Biological tests that include plants as bioindicators of contamination have become a useful tool in water risk assessment in the last few years, especially in agricultural regions. Bio-assays that involve agricultural plants as indicators are very important for the evaluation of water and sediment quality that is used for agricultural production, because the results of these studies directly show the suitability of water for irrigation and sediment for disposal on arable land (Gvozdenac et al., 2011, 2012a, 2014).
The aim of this work was to assess the quality of sediment (in the form of interstitial water) from canals Feketić, the Great Bačka canal –GBC and Stari Begej - special nature reserve "Carska bara", using chemical and biological methods, with special accent on cultivated plants as potential indicators of pollution. We also tended to estimate the potential use of these sediments in agricultural production.

Material and method

Sediment sampling. Sediment was collected during 2013 from canals Feketić, the Great Bačka canal –GBC and Stari Begej within the special nature reserve "Carska bara". At the investigated locations, composite sediment sample were taken from three deep samples. Immediately after taking, all samples were split and placed into plastic bags for the analyses. The samples were stored at 4 °C. Total concentration of particular metals was carried out according to the NEN procedures. Sediment was used in the form of interstitial water.
Phyto-toxicity test was carried out according to a standard filter paper method (ISTA, 2011) with slight modifications described by Gvozdenac et al. (2014). The most commonly cultivated plants in this area– maize (*Zea mais* L.), radish (*Raphanus sativus* L.), cucumber (*Cucumis sativus* L.) and beans (*Phaseolus vulgaris L*.) were used as test species. The effect of sediment quality on plants was evaluated according to changes in physiological (germination energy and germination - %) and morphological parameters (root and shoot length -cm and fresh and dry weight of root and shoot -g). Experiment was set in four replicates.

Statistical analysis. Data were analyzed using Duncan`s multiple range test, for 95% confidence interval, in software SPSS version 17.0 (SPSS, Inc., Chicago IL).

Results and discussion

The results of chemical analysis indicate that sediment from GBC is contaminated with Ni (46 ppb), Cu (130 ppb) and ammonium ion (460 ppm). Mentioned values exceed Maximal allowable concentrations (MAC) according to Regulation on limit values for pollutants in surface and ground waters and sediments (Official gazette, 50/12) for the II class of water. P (2504 ppb), Fe (10548 ppb) and Zn (315 ppb) are present in high amounts. Sediment from Feketić is heavily loaded with N (23 000 ppm), P (1024 ppb) and Mn (494 ppb) and from Stari Begej with Fe (7577 ppb) and Mn (247 ppb).
The results of phyto-toxicity test are presented in Table 1.
Maize. In biotest, sediment from Feketić and GBC significantly inhibited germination energy and germination of maize seeds, compared to the control (F=19.78*) while sediment sample from Stari Begej did not cause significant effects on physiological parameters of this species. Root length and fresh and dry weights of maize seedlings were significantly inhibited when treated with sediment from Feketić, GBC and Stari Begej (F=249.02**, 291.81**, 225.89**, respectively). The aboveground parts were also negatively affected by the sediment samples from Feketić and GBC (F=8.99*, 314.0**, 94.01**, p<0.01/0.05, respectively). Given indicates that maize seedlings were very sensitive to sediment pollution and total chemisam.

This is probably due to high amount of heavy metals detected in the samples which exceed MAC values. More significant inhibitions were recorded in treatments with interstitial water from GBC, which is heavily polluted with a variety of heavy metals. Maize responded in inhibition of all parameters which makes these sediments unsuitable for use in production of maize as fertilizer. Many authors have demonstrated that maize is sensitive to metal pollution, only Mahmood et al. (2005) emphasized that small concentrations of heavy metals are not responsible for *Zea mays* germination inhibition.

Radish. Germination energy and germination of radish seeds were significantly inhibited by interstitial water from sediments from Feketić and GBC, compared to the control ($F=260.80^{**}$, 260.80^{**}, $p<0.01$, respectively), while sediment sample from Stari Begej did not cause significant effects on physiological parameters of this species. Root and shoot length and fresh and dry weights of radish seedlings were significantly inhibited by interstitial water from Feketić and GBC sediment, but sediment from Stari Begej statistically significantly stimulated these parameters ($F=94.27^{**}$, 101.83^{**}, 97.89^{**}, 13.78^{*}, 242.48^{**}, 156.9^{**}, $p<0.01/0.05$, respectively). Thus only sediment originating from Stari Begej can potentially be used for irrigation of radish. However, prior to its use, it must be chemically analyzed again. Di Salvatore et al. (2008) presented different results that the presence of metals (Cu, Ni and Zn) in the substrates did not affect seed germination. However, when comparing these results the concentrations of metals present and varietal sensitivity should be taken in consideration.

Cucumber. Germination energy and germination of cucumber seeds were significantly stimulated by interstitial water from Feketić and GBC, while root length and fresh and dry weights of cucumber seedlings were significantly stimulated by interstitial water from Stari Begej ($F=23.60^{*}$, 24.21^{*}, 16.78^{*}, $p<0.05$, respectively). The aboveground parts of cucumber seedlings were also positively affected by the sediment samples from Feketić and GBC ($F=135.69^{**}$, 272.89^{**}, 97.90^{**}, $p<0.01$, respectively).These results indicate at possibility of using these sediments in the production of cucumber in the region. However, prior to application, the sediment should be re-analyzed, to avoid possible negative affects due to increase of some unfavorable substances because according to Rajić (2010) availability and concentrations of metals in sediment change seasonally. Previous findings of Gvozdenac et al. (2011) indicate that seed germination of *Cucumis sativus* L. was not affected by the chemical composition of water. On the basis of the results from bioassay, we could speculate that perhaps the presence and interaction of several compounds exceeding MAC in sediment (Cu, Zn and Ni) stimulated root elongation of cucumber seedlings. Some authors reached the different conclusions.

Beans. Germination energy and germination of beans seeds were significantly inhibited by interstitial water from Feketić and GBC sediments, compared to the control and sediment from Stari Begej which did not cause any effects on physiological traits ($F=70.80^{**}$, 80.63^{**}, $p<0.01$, respectively). Root length and fresh and dry weights of cucumber seedlings were significantly stimulated by interstitial water from Stari Begej but inhibited by sample from Feketić. Only sediment from GBC statistically significantly inhibited the above ground parts of beans ($F=26.75^{*}$, 292.13^{**}, 42.13^{**}, $p<0.01/0.05$, respectively). Only interstitial water from Stari Begej can be potentially used for irrigation of beans. However, prior to its use, it must be chemically analyzed again.

Table 1. Physiological and morphological parameters of tested plant species depending on the sediment sample quality

Parameters	Sample	Maize	Radish	Cucumber	Beans
Germination energy (%)	Feketić	97.25 ±0.25 b	95.25 ±0.25 b	98.25 ±0.25 a	90.50 ±0.50 c
	GBC	97.25 ±0.96 b	95.15 ±2.06 b	98.50 ±0.58 a	92.50 ±1.00 b
	Stari Begej	99.00 ±0.00 a	100.0 ±0.00 a	87.00 ±0.00 b	98.00 ±0.00 a
	Control	100.0 ±0.00 a	99.50 ±0.50 a	85.00 ±1.00 b	98.00 ±0.00 a
	F value	**19.78***	**260.80****	**473.91****	**70.80****
Germination (%)	Feketić	97.25 ±0.25 b	95.25 ±0.25 b	98.50 ±0.50 a	93.00 ±2.00 b
	GBC	97.25 ±0.96 b	95.15 ±2.06 b	98.50 ±0.58 a	93.50 ±1.00 b
	Stari Begej	99.00 ±0.00 a	100.0 ±0.00 a	89.00 ±1.00 b	98.75 ±0.75 a
	Control	100.0 ±0.00 a	99.50 ±0.50 a	86.00 ±1.00 b	98.25 ±0.25 a
	F value	**19.78***	**260.80****	**466.76****	**80.63****
Root length (cm)	Feketić	8.5 ±0.51 c	2.9 ±0.45 d	9.90 ±1.01 b	13.2 ±0.20 c
	GBC	7.73 ±0.36 d	4.8 ±0.36 c	10.30 ±0.53 b	14.5 ±0.87 b
	Stari Begej	10.3 ±1.02 b	7.5 ±0.43 a	13.00 ±1.09 a	15.1 ±0.08 a
	Control	12.2 ±0.25 a	6.4 ±0.51 b	10.90 ±0.89 b	14.3 ±0.52 b
	F value	**249.02****	**94.27****	**23.60***	**22.21***
Root fresh weight (g)	Feketić	2.27 ±0.25 c	0.05 ±0.004 c	0.43 ±0.03 b	2.14 ±0.22 c
	GBC	1.58 ±0.01 d	0.07 ±0.01 c	0.42 ±0.01 b	2.17 ±0.50 bc
	Stari Begej	3.62 ±0.57 b	0.21 ±0.011 a	0.54 ±0.25 a	3.23 ±0.22 a
	Control	5.33 ±0.30 a	0.12 ±0.009 b	0.48 ±0.08 b	2.58 ±0.01 b
	F value	**291.81****	**101.83****	**24.21***	**55.45***
Root dry weight (g)	Feketić	0.18 ±0.07 c	0.007 ±0.003 c	0.051 ±0.007 b	0.19 ±0.01 c
	GBC	0.09±0.002 d	0.009 ±0.002 c	0.051 ±0.01 b	0.21 ±0.08 bc
	Stari Begej	0.51 ±0.02 b	0.019 ±0.005 a	0.058 ±0.04 a	0.31 ±0.02 a
	Control	0.68 ±0.04 a	0.012±0.002 b	0.049 ±0.02 b	0.24 ±0.01 b
	F value	**225.89****	**97.89****	**16.78***	**48.76***
Shoot length (cm)	Feketić	8.9 ±0.84 bc	5.1 ±0.34 c	14.3 ±0.30 a	9.2 ±1.04 a
	GBC	8.0 ±0.39 c	5.4 ±0.40 c	13.5 ±0.40 b	7.8 ±0.76 c
	Stari Begej	9.1 ±0.14 ab	7.9 ±0.14 a	11.5 ±1.22 c	8.6 ±0.62 ab
	Control	10.2 ±0.21 a	6.1 ±0.12 b	10.1 ±0.97 c	8.2 ±0.20 b
	F value	**8.99***	**13.78***	**135.69****	**26.75***
Shoot fresh weight (g)	Feketić	4.78 ±0.02 b	0.78 ±0.10 cd	2.51 ±0.11 a	10.64 ±0.11 a
	GBC	2.53 ±0.22 c	0.65 ±0.03 d	2.21 ±0.08 b	9.15 ±1.34 b
	Stari Begej	4.99 ±0.01 ab	1.32 ±0.32 a	2.05 ±0.54 c	11.02 ±0.87 a
	Control	5.05 ±0.04 a	0.97 ±0.01 b	1.83 ±0.23 cd	10.63 ±0.61 a
	F value	**314.0****	**242.48****	**272.89****	**292.13****
Shoot dry weight (g)	Feketić	0.48 ±0.02 b	0.075 ±0.006 c	0.31 ±0.01 a	1.64 ±0.08 bc
	GBC	0.19 ±0.01 c	0.04 ±0.004 d	0.29 ±0.03 a	1.60 ±0.65 c
	Stari Begej	0.56 ±0.01 ab	0.120 ±0.02 a	0.15 ±0.07 b	2.06 ±0.56 a
	Control	0.65 ±0.04 a	0.09 ±0.013 b	0.15 ±0.01 b	1.65 ±0.13 b
	F value	**94.01****	**156.9****	**97.90****	**42.13****

Duncan`s multiple range test ±SD; values with the same letter in the column are on the same level of significance for the confidence interval 95%; **p<0.01;* 0.01<p<0.05; ns p>0.05

Results presented in this study indicate different sensitivity of tested plants to sediment quality, as well as different tolerance to heavy metals and inorganic pollutants detected in higher concentrations. This was previously reported by several authors pointing out that tolerance is species-dependant but there are also varietal differences (Ankley, 1993, Gvozdenac et al., 2011, 2012a, 2014). Sensitive species are successfully used as bioindicators of habitat contamination and for the detection of contamination in bioassays. When interpreting the results regarding plant reactions to sediment quality and contamination, the fact that metals like Cu and Zn are essential elements for plant cell development and catalyze certain physiological processes, should be considered. However, increasing concentrations in the environment can lead to morphological and physiological changes due to immobilization and inability to adopt other essential elements (Kastori and Petrović, 1997). On the contrary, Ni and Cr are noted as elements that have toxic effect on plants and are more frequent

pollutants of the environment. Also, the potential interactions of pollutants in medium should not be overlooked.

Conclusion

The results of chemical analysis indicate that:
sediment from GBC is contaminated with Ni, Cu and ammonium ion, and heavily loaded with P, Fe and Zn
sediment from Feketić is heavily loaded with N, P and Mn
sediment from Stari Begej is heavily loaded with Fe and Mn
The results of phyto-toxicity test indicate that:
sediments from Feketić and GBC had inhibitory effect on all physiological and morphological traits of maize, radish and beans
sediment from Stari Begej only inhibited some traits of maize seedlings but stimulated root and shoots traits of radish, cucumber and beans
tested sediments did not cause any toxic effects on cucumber seeds and seedlings, even stimulated certain parameters
due to sensitivity expressed in this assay, maize can be considered as good indicator of sediment quality and contamination with metals detected in high amounts (Ni, Cu, Fe, Zn, Mn)
The overall results indicate at possibility of using these sediments in the production of cucumber in the region. However, prior to application, the sediment should be re-analyzed, to avoid possible negative affects due to increase of some unfavorable substances

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REDUCING THE NICOTINE CONTENT THROUGH TOBACCO WASTE COMPOSTING WITH OTHER ORGANIC MATERIALS

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Abstract

The aim of this research was to investigate the tobacco waste composting with addition of different organic materials. Tobacco waste (TW) represents significan percent of industrial and communal waste in Serbia. If the nicotine content in tobacco waste is greater than 500 ppm of dry weight, according to European Union Regulations, this waste is classified as „toxic and hazardous”.

Virginia tobacco leaves, which remain after processing, were used as a waste. Two experimental samples are formed according to the following scheme: I 60% TW + 40% cabbage; II 60% TW + 10% soil +15% dry forest leaves + 15% cabbage. The comparison was made with the reference sample (only TW).

Experiment was set up in the indoor area at the Faculty of Agriculture, without automatic control of microclimatic parameters. The indoor area is periodically re-heating by electrical heater, and the relative humidity is controlled only by natural ventilation. During the process of composting, temperature and humidity in all samples and in the room was measured. Nicotine content in samples was determinate by HPLC method.

Initial nicotine content in tobacco waste was 17 363 mg/kg. By adding other organic material to the tobacco waste, nicotine content was decreased in the range of 37.5 – 46.5%. At the end of the research, nicotine content has dropped below 10 ppm in both experimental samples. Reduction of the nicotine content showed that organic materials can be used in the composting of hazardous tobacco waste.

Keywords: *tobacco waste (TW), Virginia, organic material, composting*

Introduction

A growing concern for the environment has resulted in the development of new technologies, materials and ways to reduce the amount of waste and environmental pollution in general. Increasing environmental awareness in recent years caused an increase in interest in the composting process in order to prevent or reduce environmental pollution, as well as sustainable management of waste generated as a product of the various activities of society (Jovičić, 2006).

Composting is the biological decomposition of organic matter under the influence of microorganisms in aerobic conditions. The composting process leads to stabilization of biodegradable waste (wet and solid organic matter, food waste, garden waste, paper, cardboard, etc.), in order to finally obtain a stable product, compost (Jovičić, 2006). This process can be conducted in a controlled, partly controlled and uncontrolled environmental condition.

In Serbia, agriculture is one of the major industries, so that generates large amounts of waste. The largest part of agricultural waste in the Republic of Serbia makes corn stalks, wheat straw and then the remains of sunflower (Jovanović and Parović, 2009). Tobacco waste, which is generated in all processes, from tobacco leaf production to the production of cigarettes, represents a significant percentage of agro-industrial and municipal waste in Serbia

(Radojičić et al., 2009). His elimination and/or recycling are mostly left to the initiative of individual companies for tobacco processing and manufacturing.

Tobacco waste includes waste remaining after breeding, processing and manufacturing of tobacco, but also after the consumption of tobacco products (cigarette butts, wrappers, cartons). The total global tobacco (Nicotiana tabacum L.) waste production in the year 2005 was more than 25.1 million metric tons (Jun et al., 2010).

According to Waste Catalogue issued by the Ministry of Environment and Spatial Planning and the Agency for Environmental Protection of the Republic of Serbia (Waste Catalogue, 2010), tobacco is classified as non-toxic waste. Although this waste is not classified as dangerous, it is not categorized as inert and therefore cannot be disposed of to landfill without treatment (Radojičić et al., 2009).

The main characteristics of the tobacco waste are a high value of the total organic carbon (TOC) and also a high content of nicotine, which is a toxic compound (Civilini et al., 1997). Its water fraction has a concentration of TOC above 200 mg/l and therefore cannot be disposed of in a sanitary landfill. According to the EU Directive nicotine content over 500 ppm declares this waste in the category of hazardous waste. Also, due to the nicotine high solubility in water, there is a serious risk in preservation that nicotine can be flushed out of the trash and end up in the groundwater, which is a particular dangerous for the environment and human health. For these reasons, tobacco waste cannot be disposed of together with urban waste (Pichtel, 2005), and must be destroyed under special conditions, which has a great economic impact for the tobacco producers and processors.

Tobacco waste recycling, or its preparation and reuse are very important, both because of the economic effects, but also due to the reduction of pollution or degradation of the environment (Novotny and Zhao, 1999; Okur et al., 2008).

Tobacco waste can be managed in the same way as other agricultural waste, by aerobic composting degradation (Felicita et al., 2003). Composting creates a stable product that can be used as an organic fertilizer.

The aim of this study was to examine the possibilities of tobacco waste composting with the addition of various organic waste materials, with the purpose of nicotine biodegradation until receiving the product that can be used and which is not dangerous for the environment.

Materials and methods

Virginia tobacco leaves, which are declared as waste after redrying process, were used for the purposes of the experiment. As components in experimental batches were used dry leaves of trees and soil, which were collected in the park around the Faculty of Agriculture, University of Belgrade and cabbage waste that is commercially available. Experiment was carried out during the period of three months: December 2014 - March 2015 at the Faculty of Agriculture, University of Belgrade. Experiment is carried out in several stages:

Preparation of experimental batches - Low humidity is one of the main characteristics of tobacco waste. Humidification was the process carried out in the first phase of experiment, and after that grinding and mixing tobacco with other biodegradable waste was performed. For the purposes of the experiment the following compost batches were formed (Table 1):

Table 1: Composting batches composition (%)

composting batch	TW	soil	cabbage	dry leaves of trees
1	60	/	40	/
2	60	10	15	15
control	100	/	/	/

Monitoring parameters of the composting process - Experiment was performed in a closed room, which is not equipped with devices for the automatic control of microclimate parameters. For this reason, the room periodically heated with electric heaters and the relative humidity is regulated only by natural ventilation.

During the experiment, in a period of three months, measuring the temperature and humidity in all samples was done every day (K-type thermometer 5115; microprocessor-moisture meter, model No. RS7825PS, CE ISO-2178), as well as temperature and relative humidity of the room (mini weather station Kestrel 4000, Nielsen-Kellerman).

Analysis of nicotine content in samples of experimental batches - Nicotine content in batches during the composting process was determined by High Performance Liquid Chromatography method (HPLC, Waters Breeze, USA).

The method applied in this analysis involves water extraction of the milled material followed by separation of the alkaloids on reverse-phase C18 column with a mobile phase of 40% methanol containing 0.2% phosphoric acid buffered to pH 7.25 with triethylamine. This procedure allows the quantitative analysis of four "major" alkaloid in tobacco and may be partially automated way to handle a larger number of samples (Saunders and Blume, 1981).

Results and discussion

Table 2 shows the results of nicotine content in experimental batches.

Table 2. Nicotine level in experimental batches (mg/kg)

	10.12.2014	**26.12.2014.**	**15.1.2015.**	**31.1.2015.**	**15.2.2015.**	**3.3.2015.**	**18.3.2015.**	**3.4.2015.**
R	17 363,0	13 064,5	11 234,2	9 515,3	8 796,3	6 902,0	5 680,0	5 132,0
1	9 280,0	5 426,9	5 108,8	4 060,4	1 138,8	208,2	<10	<10
2	10 860,0	9 725,1	6 768,6	867,7	393,3	118,6	<10	<10

R – Control sample

The initial content of nicotine in tobacco waste was 17 363 mg/kg. Nicotine content was reduced by 46.5% in sample 1 and 37.5% for the sample 2 at the beginning of the experiment, mixing with other forms of organic waste.

Both experimental batch showed solid results in the degradation of nicotine. At the end of the experiment, after three months (the results of 18.03.2014.), nicotine content has fallen below 10 mg/kg in both experimental batches. In order to check the effectiveness of the experiment, after 15 days, it was performed another analysis of nicotine content in the samples.

Although the process of composting was carried out successfully in both experimental batches, the sample 2 showed better results in the nicotine degradation. In the sample 2 (addition of 15% dry leaves of trees + 10% soil + 15% cabbage waste) nicotine degradation was occurred below the limit of 500 mg/kg (393,3 mg/kg) after two months from the experiment beginning. It took 15 days to decrease the nicotine content below 500 mg/ kg in the sample 1.

The results of this experiment, which refer to the nicotine content, confirm that the organic material, which also presents the waste, can be successfully used for the composting process of tobacco waste.

During the experiment, the dynamics of nicotine decomposition depended of the temperature and the humidity in the experimental samples. From the results shown in Table 2 it is possible to conclude that the increase in temperature and humidity patterns and dynamics of decomposition was higher, especially in the second half of the experiment. Temperature and humidity were measured daily. For better understanding of process, the results given in Figure 1 and Figure 2 are the average values of the process parameters.

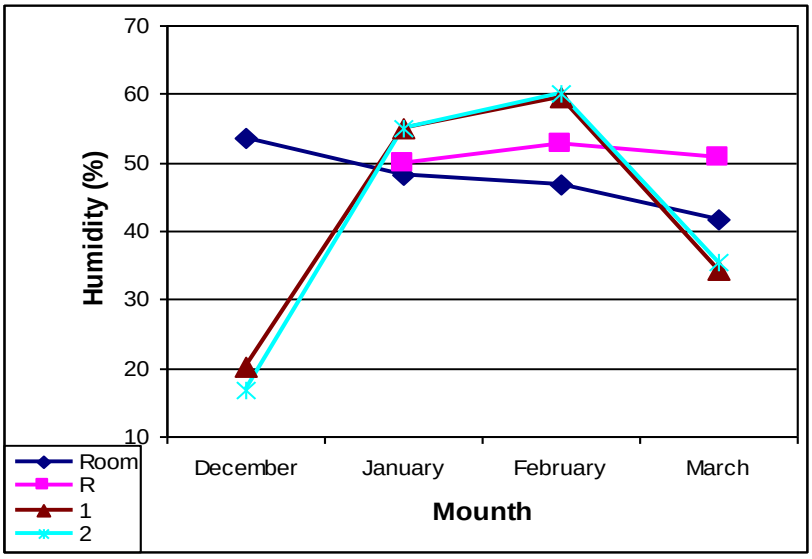

Figure 1. Humidity mean values during the composting process

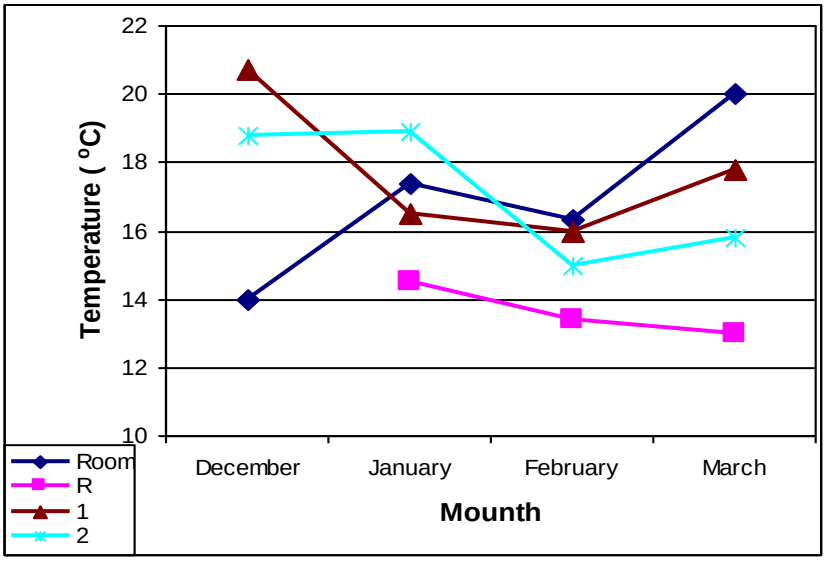

Figure 2. Temperature mean values during the composting process

With the progression of the composting process, it was observed that humidity had risen in experimental batches, due to intensification of the process. On the basis of the average

moisture content shown in Figure 1, it can be concluded that both experimental samples (1 and 2) had almost identical values. Sample 1 had a higher temperature at the beginning of the experiment, so the speed of nicotine degradation was higher at the beginning. After that, due to the rapidly temperature decreasing in the batches, speed of nicotine degradation in the second month of experiment was reduced. In the sample 2, in the first month of experiment, temperature was maintained at constant value, which resulted in a faster degradation of the nicotine in the later stages.

Low air temperature in the experimental room has slowed down the process of biodegradation. If the composting process would be conducted under controlled conditions, with automatic regulation of parameters, this could lead to significantly shorter process of duration.

Conclusion

Tobacco waste can be used as fertilizer due to its high content of organic matter. But first, it needs to be made applicable because in its original form it's still toxic. Effective implementation of the nicotine biodegradation of tobacco waste has great importance, both in terms of efficiency and maximum utilization of tobacco raw material and environmental protection.

From the presented results it can be concluded that the dynamics of acceptable nicotine degradation can be achieved by mixing the tobacco waste with other types of organic waste (cabbage, dry leaves of trees and soil). Therefore, by mixing tobacco waste with other types of organic waste, the nicotine content had already decreased in the range of 37.5 - 46.5% at the beginning of the experiment. At the end of the experiment nicotine content has dropped below 10 mg/kg in both compost batches, which is far below the values prescribed by the EU Directive.

Results of the experiment showed that it is possible to perform nicotine biodegradation of tobacco waste in order to archieve non toxic organic material that can be used without the risk of environmental pollution.

The importance of this experiment is the fact that the process of biodegradation can be carried out in natural conditions, regardless of the process duration, and it does not require a great economic investment. If the composting process would be performed in the summer months with high outside temperatures and adequate relative humidity, the natural process would be faster. In this case, available forms of organic waste in this period of the year must be taken in account. On the other hand, if the composting process would be carried out in controlled conditions, with automatic regulation of parameters, the duration of process would be significantly shortened. These conclusions open up opportunities for further research.

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RESEARCHES ON DEMOGRAPHIC VARIATION OF NEW ARTIFICIAL YANARDÖNER (Centaurea tchihatcheffii Fisch. & Mey.) POPULATIONS

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Abstract

Yanardöner (Centaurea tchihatcheffii Fisch. & Mey.), belong to Asteraceae (Compositae), is an annual endemic plant species and localized in an extremely narrow arable fields in Gölbaşı, Ankara, Turkey. C. tchihatcheffii, ranks among Critically Endangered Category with respect to IUCN criteria due to under pressure agricultural practices and urbanization. Some agricultural practices significantly affected natural populations, such as using herbicide, tillage and burning stubble. Creating new populations of narrowly endemic plant, like C. tchihatcheffii, in a suitable place protected from the outer pressure is an approach to protect of the plants. Demographic variations of new artificial population in the field conditions were keenly observed through the three years.

Field experiments were conducted in the Yenimahalle Agriculture Campus (YAC) to determine the establishment ability of C. tchihatcheffii population in a new area and the demographic structure of new population in the YAC. The factors that have influence on the population growing, such as the seedling time and number, the mature plant number and blossoming time, the maturation time of seeds and the seed numbers reach to the soil at the end of the season, were determined as the part of the demographic studies. The population established with 10 seeds per square meter in the soil reached considerable amount of seed number (2792 seed m-2) at the end of the trial. Death ratios of the germinated seeds were 11.7% at the cotyledon stage, 30.9% at the seedling stage and 5.6% at the blossoming stage. The rate of seed maturation ranged from 0.5 to 86.7% depends upon climatic conditions. Severe drought occurred after emergence of the seeds caused to decline about 99.5% of the population.

Keywords: *Demographic variation, Centaurea tchihatcheffii, artificial population, narrowly endemic plant*

Introduction

Turkey has hosted more than 10,000 plant taxa, approximately one-third of them is endemic. This high ratio of endemism can be explained by the variety of climate, geomorphological and soil diversity, the situation of the area at the junction of three flora region (Euro-Siberian, Mediterranean and Irano-Turanian) and ecological factors, and made Turkey a rich country for endemic taxa (Avcı, 2005; Davis, 1982). Asteraceae comprises 1,535 genera with 26,000 species in the world while it is represented by 1209 species in the Flora of Turkey, out of which 447 are endemic, with an endemism of 37% (Doğan et al., 2009). Among 134 genera of Asteraceae in the flora of Turkey, *Centaurea* L. is described as one of the largest genera (Wagenitz et al., 2005).

Centaurea tchihatcheffii (Fisch. & Mey.) is an annual endemic plant species and localized in an extremely narrow arable fields in Gölbaşı and Gölbek Village, Ankara, Turkey (Çakaroğulları, 2005; Vural, 2009). *C. tchihatcheffii* ranks among the Critically Endangered Category with respect to IUCN criteria due to herbicide applications and collection for ornamental purposes under pressure agricultural practices and urbanization (Ergüner-Baytok, 2008). Some agricultural practices significantly affected natural populations, such as using

herbicide, tillage and burning stubble. Creating new populations of narrowly endemic plants, like *C.* tchihatcheffii, in a suitable field protected from the outer pressure, is an approach to protect of the plants.

Conservation strategies of endangered species can be evaluated through two basic strategies called in-situ and ex-situ conservations (Brutting et al., 2013). In-situ conservation is a technique used for maintaining the viability of the populations in their natural habitats such as, national parks and gene management zones (Ergüner-Baytok, 2008) while ex-situ conservation is mostly used to be applied to the populations outside of their habitat in artificial conditions such as, botanical gardens, game farms, and gene banks (Primack, 1999). Ex-situ conservation encompasses not only the advantage of supplying new habitats for endangered plant species, but also it has some risks to adapt the species, such as genetic erosion, artificial selection and spontaneous hybridization (Volis et al., 2009). *C. tchihatcheffii* has also potential economic value as an ornamental plant because it has got remarkable flowers with shiny pinkish red (Ergüner-Baytok, 2008). Therefore, ex-situ conservation studies will contribute to maintain the viability of the species and to breed for ornamental purposes.

Some individual efforts have made to establish *C. tchihatcheffii* populations in protected areas, and they have promising results for the longevity of the species. The species have been established in Nezahat Gökyiğit Botanical Garden (İstanbul/Turkey) and successfully adapted in there (Vural, 2009). Savcı, (2007) also found that the species can survive in the protected areas where they have different type of soil. Demographic variations of *C. tchihatcheffii* populations in a new field condition were keenly observed through the three years to determine the establishment ability of new artificial populations.

Materials and Methods

The study on the establishment of the *C. tchihatcheffii* populations was performed in YAC 10 stable plots, each size was 1 m^2 (1m x 1m), were arranged on the two rows, and there was 0.5 m between adjacent plots. The seeds were sown in a depth of 2.5-3 cm in the late of December 2004 as a first step of 3-year study. Each parcel contained 10 matured seeds. Afterwards, the plots in YAC were keenly observed at 15 days intervals. The plants have been checked from the rosette stage to the mature plants. The parameters was monitored during the life cycle of plants were germination, mortality, phenology, achene output per plant, life stage transitions, fecundity and the number of seed included to the seed bank. Calculation of the plant's contribution to the seed bank in a plot was predicted by multiplying the number of mature capitulum by the average seed number of each capitulum.

The influence of seed burial depth on seed germination was performed during the trial in YAC due to the fact that the seed longevity of *C. tchihatcheffii* within the seed bank had not been known. Trial was conducted using the seeds collected from Gölbaşı in 27.05.2004. The seeds/sand mixture which were placed in nylon fabric bags were sown at depths of 5, 10, 15 and 20 cm in the clay pots. The efficacy of dry storage on seed germination was also carried out. For this aim, the seeds were put inside paper envelopes were stored at +5^{o}C during the same durations. The seeds sown at varies depths were exhumed of 1, 6, 12 and 24 months to determine germination rates in the laboratory conditions. At the same time, the seeds stored were taken off paper envelopes for the same purpose. The experiment was conducted according to the Rules of International Seed Testing Association using a total of 400 seeds (ISTA, 1985). The petri dishes were incubated at constant temperatures of 10°C and 12 h/12 h of light/dark photoperiod cycle to determine the effect of burial at various time on the germination percentages while the dishes put inside a growth chamber at constant temperatures of 5^{o}C and 10°C and darkness and 12 h/12 h of light/dark photoperiod cycle to determine the influence of dry storage duration on the germination percentages for 30 days.

Germinated seeds were counted and removed from petri dishes at 2-day time interval, and water was added as needed. Experiment was conducted with the use of a completely randomized design with four replicates. Data were subjected to ANOVA (α=0.01).

Results and Discussion

The biological cycle of *C. tchihatcheffii* in YAC, from the rosette to the mature plants, were keenly monitored from 2004 to 2007. Growing stages of the plants launched when the rosettes were appearance on the soil surface at the early of December. First rosettes were seen in plots 10-12 days after an effective rainfall was occurred. Although many of seeds emerged in December, it was observed that emerging of a small number of seeds continued in the spring months due to the irregularity of autumn rainfall. Rosette, flowering and maturity dates of populations in YAC are given in Table 1. The flowering stage followed emerging stage, heavily occurred in October, and continued from early of April to early of June (30-45 days). Maturity of the plants started at the late of May in 2006 and the second week of June in 2005 and 2007. Variation at the duration of biological stages can be caused by the climate, especially the amount of rainfall and its time. These results are in harmony with those of Çakaroğulları (2005) and Savcı (2007) except some little differences regarding the emerging, flowering and maturation times. These discrepancies may be related to climate and soil conditions.

Table 1. Emerging, flowering and maturation dates of *C. tchihatcheffii* from 2004 to 2007

Year	Emerging Date	Flowering Date	Maturation Date
2004–2005	15.01.2005-15.04.2005	25.04.2005-01.06.2005	08.06.2005
2005–2006	10.10.2005-15 02.2006	01.04.2006-15.05.2006	30.05.2006
2006–2007	01.10.2006-15.11.2006	15.04.2007-15.05.2007	11.06.2007

As it can be seen in the Table 2, more than two of three of seeds sown emerged in the first growing season (2005); however, emergency ratio of the seed in the plots approximately 2% in the second growing season (2005-2006) and 3.2% in the third growing season (2006). There was a huge disparity between the results of the first season and the second-third seasons, which can be caused by more number of immature and undeveloped seeds. Viable seed ratio of *C. tchihatcheffii* in natural seed banks were determined in 2005 using the soil sample taken from natural habitats of *C. tchihatcheffii*. This ratio was 25.37% in a natural habitat where didn’t any agricultural practise while the soil in cultivated field had high viable seed ratio (65.85%). Many researchers have found that using fertilizer, fine tillage of soil and weather conditions can improve seed viability (Jevdovic et al., 2013; Hay and Probert, 2013; Sveinsson and Björnsson, 1994) like *C. tchihatcheffii*.

Table 2. Settlement of *C. tchihatcheffii* in YAC

Plot	Seed Bank	Budding density (Plant number m^{-2})			Mature plant Density (Plant number m^{-2})			Seed Number included to seed bank			Sum
		2004-2005	2005-2006	2006-2007	2004-2005	2005-2006	2006-2007	2004-2005	2005-2006	2006-2007	
1	10	5	5	25	10	0	3	182	0	195	
2	10	4	32	90	10	31	1	650	1274	39	
3	10	6	-	39	10	-	0	1547	-	0	
4	10	10	11	61	10	9	1	1196	507	39	
5	10	5	90	60	10	83	0	1235	463	0	
6	10	9	13	117	10	9	0	5434	923	0	
7	10	6	102	179	10	28	0	1508	4875	0	
8	10	7	48	100	10	48	0	2067	689	0	
9	10	9	21	75	10	10	0	1495	819	0	
10	10	7	29	170	10	21	0	2236	1781	0	
Sum	**100**	**68**	**351**	**916**	**100**	**239**	**5**	**17.550**	**11.331**	**273**	**27.919**

The seed bank was established by starting with 10 matured seeds per square meter, and the number has reached to 2.791,9 seeds averagely at the end of three growing season (Table 2). Although there was a steady increase in the population size in two-years, a sudden decrease of the population size was observed in 2007. These dramatic declines were caused by severe drought in 2007 and more than 99% of budding couldn't reach maturity. Average seed number to include seed bank was calculated multiplying by the capitulum number of the mature plant and average seed number in each capitulum (13 seeds per capitulum). Calculation was only done using the plant emerged and matured because non-emerged seeds couldn't be observed. Approximately half of plants died before they reached maturity (Table 3). The highest mortality rate was observed at the budding stage of *C. tchihatcheffii* (30.9%). A study carried out by Çakaroğulları (2005) in Gölbaşı/Ankara (Natural habitat) reveal that percent mortality at rosette stage and budding stage ranged from 2.02 to 18.35 and from 1.39 to 6.06, respectively. These percent mortalities are very low compared with our results. This contrast may be related to natural environment because enemies in new habitats can be more destructive than natural habitat due to limited individual plants.

Table 3. Mortality ratio of *C. tchihatcheffii* at various growing stages

Year	Mortality Ratio (%)			Sum (%)
	Rosette	Budding	Flowering	
2005	-	8.8	4.5	13.3
2006	17.7	11.7	2.6	32
2007	17.5	72.3	9.7	99.5
Average	11.6	30.9	5.6	48.3

The efficacy of dry storage on seed germination was determined with a-24 month assay. Seed germination exhibited a steady increase depending on time (Figure 1). Although the germination rates were low initially, at the end of trials (24th month) the germination ratios were higher than 80%. No differences was found among germination rates in the darkness and in the dark-light cycle at 4 storage times ($P>0.01$). This results suggest that light is not required for *C. tchihatcheffii* seed germination, and the differences among germination rates at 5^oC and 10^oC are not significant statistically ($P>0.01$). The difference between germination rates at 4 storage durations are significant statistically ($P<0.01$).

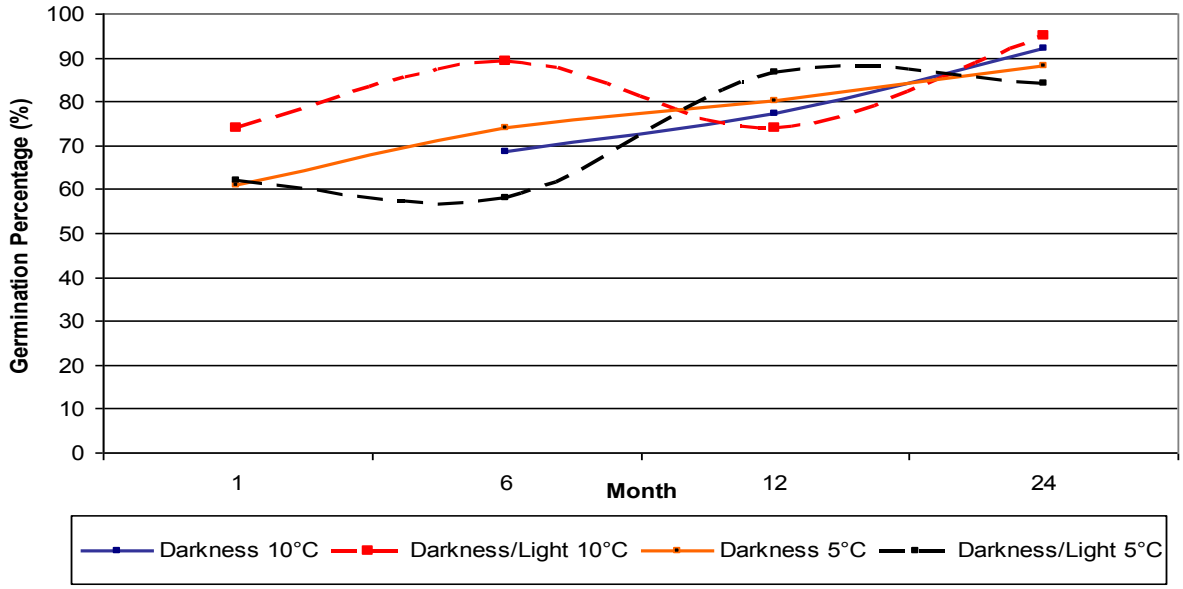

Figure 1. The influence of dry storage duration on the germination percentages of *C. tchihatcheffii* seeds

Seed are exposed the effects of many biotic and abiotic factors, such as microorganisms, pests, water, salt and pH when they included into soil. These factors influence viability of seed, and germination percentage are getting down day by day. Germination percentage of the seeds was influenced by seed burial depth (Figure 2). This percentage was higher for seeds placed at near the soil surface than for seeds buried at more depth. Statistical analysis of trial results has demonstrated that interaction between Time and Burial Depth is significant ($P<0.01$). Decreasing of germination percentage was very high compared with the results obtained from the seeds 1 month stored in dry conditions (Figure 1). This sharp declining can be caused by secondary dormancy, is induced by unfavourable environmental conditions. Some reports have indicated that extreme temperatures and high soil moisture significantly influence the occurring secondary dormancy (Anderson and Milberg, 1997; Jursík et al., 2010). Our findings are in agreement with the previous studies, buried *Bifora radians* seeds couldn't germinate at favourable environmental conditions in laboratory if the season wasn't suitable to her (Taştan, 1988) and duration and burial depth has got negative effect on the viability of *Boreava orientalis* seeds (Taştan et al., 1997).

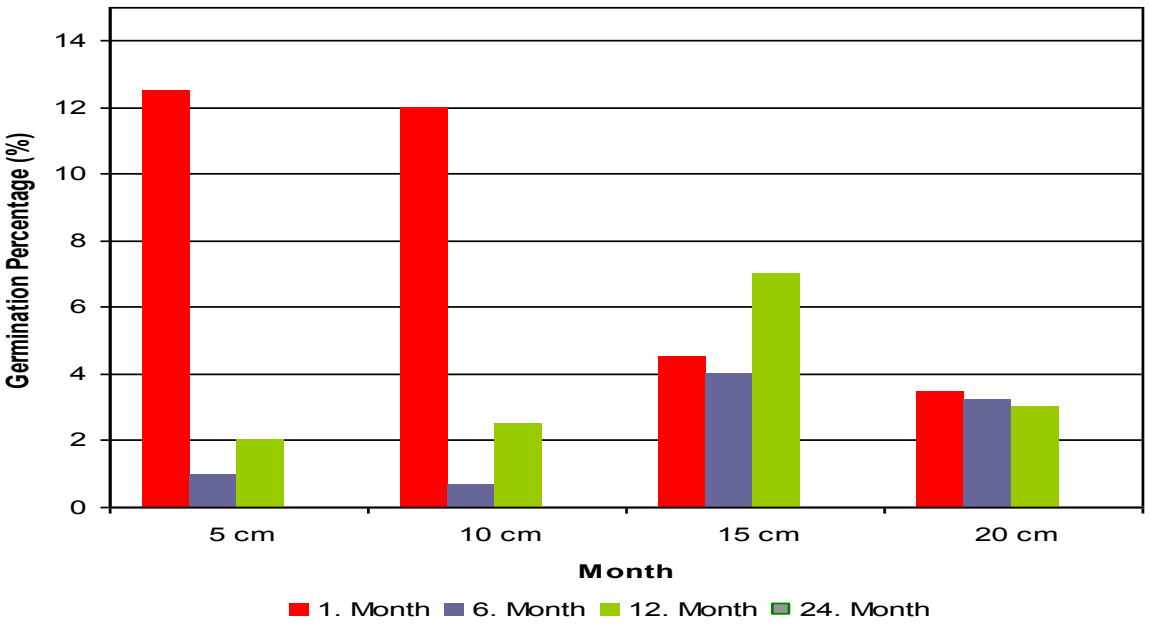

Figure 2. The effect of burial at various times on the germination percentages of *C. tchihatcheffii* seeds

Conclusion

The creation of new populations is one the most important solution to decrease the risk of extinction of *C. tchihatcheffii*. Our results indicated that *C. tchihatcheffii* can easily settle in the new habitats although the populations more vulnerable to outer factors compared with natural habitat. The present study also confirmed that the new populations may create strong seed banks for establishment of viable populations. These seed banks are the key factor to generate the new offspring and for population viability. Although the study finished eight years, the individuals of *C. tchihatcheffii* have been seen in study area. New populations and also seed banks for *C. tchihatcheffii* should be created at some protected areas if anthropogenic pressures on the populations continue in its natural habitats. Production of *C. tchihatcheffii* for ornamental purposes in new cultivation fields not only support the longevity of the species, but give an economical opportunity for florist as well.

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Review paper
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THE EFFECTS OF THE OZONE GAS PRODUCED IN GENERATOR ON THE ENVIRONMENT, FOOD AND HUMAN HEALTH (RECENT DEVELOPMENTS)

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Abstract

Nowaday, the increasing importance given to human health, and the impact of legislation prepared for it, more environmentally friendly clean residue-free disinfection methods have been developed. One of these applications is ozone application. Ozone in its gaseous form kills insects in various commodities and this activity has been thoroughly evaluated by many researchers under laboratory and field conditions, with various application techniques. But oxidation of ozone gas does not usually result in complete mineralization, but rather in the formation of oxidation by-products (e.g. aldehydes, ketones, alcohols, bromate and more) can also be generated from the reaction of ozone with according to the type of contaminants. Ozone treatments on studies have recently focus on kept at allowable value of these by-products will be formed, increasing the effect of ozone, ensuring more economical production and more. This review provides answers to the following questions: What is the level of effectiveness of generated ozone gas? What are the effects on the environment, food and human health produced ozone gas? Is there any advantage from other disinfection methods? Is it true that ozone gas some of the claims of destroying bacteria and viruses types, and which extends the shelf life of the food? Lastly, which combine methods can be increased the effectiveness of ozone gas?

Keywords: *Ozone gas application, desenfection, food, human health.*

Introduction

Ozone, the triatomic form of oxygen (O_3), is highly reactive and a strong oxidizing agent, which is classified as “GRAS” (Generally Recognized As Safe) by the United States Environmental Protection Agency (FDA, 1982). To be used in bottled water and in food processing (Graham, 1997). Moreover, the Food and Agriculture Organization (FAO, 1994) recognizes the potent disinfectant characteristics of O_3.

Ozone is toxic to all forms of life, and governmental regulations have been established to protect people from excessive exposures to this gas. This reason, the effects of ozone treatment on the quality and physiology of the commodities mainly depends on ozone doses, exposure time and environmental conditions such as treatment medium and temperature.

The human threshold limit values vary from 60 to 100 parts per billion (ppb) in air, depending on the agency or country involved. Ozone can be generated by exposing normal diatomic oxygen gas to energetic electrons, X-rays, nuclear gamma rays, short-wavelength ultraviolet radiation (UV) and corona discharge (electrical field) and more recently by electrochemical methods. Other disinfection methods outside the ozone gas application are ultraviolet light, ultrasound, electrolyzed oxidized water, high pressure carbon dioxide, and bacteriophage. Other novel technologies like high pressure processing, pulsed electric field processing and irradiation were not covered considering their vastness of literature and availability of published reviews (Mukhopadhyay and Ramaswamy, 2012).

Throughout the world, ozone has been used to purify drinking water, kill bacteria, sanitize food, deodorize, decolorize and decrease some contamination. The modern life-style of developed countries involves daily usage of artificial compounds such as active

pharmaceutical (such as human or veterinary) ingredients (API), personal care products, hormones, pesticides or biocides and other environmentally persistent chemicals. Many of these potentially harmful substances are not biodegradable and do not adsorb easily. As a result residues of these compounds become micropollutants in wastewater which leads to an insufficient elimination of them in wastewater treatment plants. During ozonation most organic micropollutants present in treated. Another source of wastewater is grey water has a huge potential to be treated, recycled and reused, especially when the availability of freshwater is a concern in many countries. (Oh et al., 2015). Wastewater are oxidized either by a direct reaction with O_3 or by secondarily formed hydroxyl radicals ($^{\cdot}OH$). However, undesired oxidation by-products from the oxidative transformation of matrix components can also be formed. The oxidative transformation of dissolved organic matter (DOM) leads to assimilable organic carbon (AOC) or biodegradable organic carbon (BDOC), which includes compounds such as aldehydes, ketones or organic acids. These compounds are typically removed during a subsequent biological treatment step. The oxidation of trace organic contaminants and potential microbial inactivation must be balanced with the formation of disinfection by-products, including bromate. However, during ozonation of saline waters such as sea water highly toxic and persistent by-products are formed, as ozone reacts rapidly with selected halogen ion such as iodide and mainly bromide to different secondary oxidants summed up as ozone-produced oxidants (OPO). It has been shown that brominated oxidants such as hypobromous acid/hypobromite($HOBr/OBr^-$). Same way, the carcinogenic N-nitrosodimethylamine (NDMA) can be formed during ozonation of wastewater from effluent organic matter or micropollutant precursors. Also, seawater electrolysis is also efficient in generating chlorine ($HOCl/OCl^-$). It is expected from a disinfection process both should provide sterilisation and by-product should not form as well as or remain within acceptable limits. Acceptible limits of bromate concentrations for ozonated wastewaters were above the drinking water standard of 10 mg L^{-1} (WHO, 2005). And the NDMA guideline value for drinking water of 100 ng L^{-1} given by the World Health Organization (WHO, 2008).

In order to increase the impact of ozonation process, reduce the formation of by-products, and reduce the cost of disinfection, scientists are trying to develop the different combine method can be used together with ozone gas. In this study has been mentioned developed in some combined methods.

Water and food desenfection application

The extensive use of Bisphenol A (BPA) in the plastics industry has led to increasing reports of its presence in the aquatic environment. Ozonation, have been shown to effectively degrade BPA(Umaret al.,2013). Ozone caused a decrease in particle stability and dissolved calcium concentration at pH of 6 (Sadrnourmohamadi and Gorczyca, 2015). Antoniou et al. (2013) investigated the ozone dosage required to remove active pharmaceutical ingredients (APIs) from biologically treated wastewater. 15 of the 42 investigated APIs could be classified as easily degradable ($DDO_3/DOC \leq 0.7$). Fenitrothion and deltamethrin insecticides are widely used on wheat grains in the storage units. The residues of fenitrothion were decreased after direct O_3 gas treatment for 180 min of exposure (Savi et al., 2015). Spices and herbs often contaminated with pathogenic bacteria and fungi. The results demonstrated that the gaseous ozone treatment is an effective alternative microbial reduction technique for dried oregano (Torlak et al., 2013). The ozone generation system is capable of reducing Escherichia coli O157:H7 in packaged spinach (Klockow and Keener, 2009). Segat et al.(2014)’s study demonstrates that water acting as carrier of spoilage icroorganisms can be successfully treated with ozone in order to reduce microbial cheese contamination in each processing step after stretching and shaping. Microbial growth and darkening rate of fresh noodles made from ozone treated flour were delayed significantly (Li et al.,2012).

Medical application and other applications

Medical ozone therapy is used for the treatment of inflammation, infected wounds, chronic skin disease and advanced ischemic illnesses, including burns, due to its antioxidant, antiflammatory and antimicrobial effects. Additionally, Ozone therapy has been successfully used in the management of diabetic foot, lumbar disc herniation, and vascular disorders, including myocardial infarction and stroke. The other techniques to supply O_3 therapy are rectal, vaginal, and ear insufflations; injection to veins, joints, muscles, and tumors; and bagging of the use of ozonated water and oil orally and externally(Case Report, 2015). Ozone was demonstrated to be a reparative substance against acoustic trauma and, in addition, it can be supplied and applied easily(Lopes et al., 2015). However, pure ozone is not used in ozone therapy owing to ozone toxicity. It is applied in the form of an ozone/oxygen mixture (Yenigun et al.,2013). Ozone might be a useful tool to reduce and control oral infectious microorganisms in dental plaque and dental cavity. However, Almaz and Sönmez, (2015) in their review study reported that the results of in vitro studies are controversial; while some researchers reported that ozone therapy had a minimal or no effect on the viability of microorganisms (Müller et al., 2007; Hauser-Gerspach et al, 2009), others suggested ozone to be highly effective in killing both gram-positive and gramnegative oral microorganisms (Holmes, 2003; Baysan and Lynch, 2004). In one study forty-four patients had intradiscal injections and were included in the analysis. After treatment, 75.0% showed significant improvement in pain based on the visual analog scale with using ozone (Murphy et al., 2015). Similarly, Teixeira et al.(2013), in their study on dogs, ozone had no measurable adverse effects and is an alternative option to promote pain relief. Ozone oxidative preconditioning attenuates experimental contrast-induced nephropathy. This effect is suggested to be mediated by reinforcement of renal antioxidant defenses and maintenance of renal nitric oxide levels by Kurtoglu et al. (2015). were shown to be effective in increasing bone healing using of hyperbaric oxygen and ozone, separately and in combination.

Zhang et al. (2011), in their review correlated different air cleaners (high efficiency particulate air (HEPA), adsorption, ultraviolet germicidal irradiation (UVGI), photocatalytic oxidation (PCO), thermal catalytic oxidation (TCO), plasma, botanic air cleaners, ion generators, and electrostatic precipitators). They concluded that none of the reviewed technologies was able to effectively remove all indoor pollutants and many were found to generate undesirable by-products during operation. Furthermore, particle filtration and sorption of gaseous pollutants were among the most effective air cleaning technologies, but there is insufficient information regarding long-term performance and proper maintenance. Finally, ozone is not recommended by Zhang et al. (2011) for indoor air cleaning because of the harmful by-products.

Method comparisons

Aday and Caner (2014) examined ozone and chlorine dioxide, alone and in combination, on strawberry storage life. Overall their findings suggest that combinations of ultrasound plus ozone and chlorine dioxide could be used for prolonging shelf life of strawberries. In the other study examined the effects of sonication, sanitizers and sodium dodecyl sulfate (SDS) on the quality of fresh-cut Iceberg and Romaine lettuce. As a result reached chlorine-treated samples had a significantly higher overall quality score than that from the other treatments(Salgado et al., 2014). In different reserch suggested by Crowe et al. (2012) that ozone treatments resulted in the greatest log reductions in mesophilic bacteria, yeast, and mold population. This impact was short-lived and only observable at time. The greatest microbial reductions in all populations were observed on chlorine-treated berries. On the contrary, in Olmez and Akbaş (2009)’s study, ozone treatment was found to be better than the chlorine and organic acid treatments in maintaining the sensory quality on Listeria monocytogenes counts and the

overall visual quality of lettuce. For compounds with low reactivity toward ozone, ozonation treatment could be insufficient in removing them from drinking water, therefore hydroxyl radical-based treatment techniques such as O_3/H_2O_2 or UV/H_2O_2 are recommended by Jin et al.(2012). NDMA(N-Nitrosodimethylamine) formation potential was significantly reduced by ozone and ozone/peroxide pretreatment (Pisarenko et al, 2012).When the comparison of the UV-based advanced oxidation processes (AOPs) VUV, UV/O_3 and UV/H_2O_2 under the experimental conditions, the combination VUV/O_3 is a good alternative to the other UV-based AOPs since it provides an economic treatment of raw waters containing taste and odour compounds and the minimization of undesired by-products (Zoschke et al., 2012). In the other study on O_3, UV, and O_3–UV were effective disinfection treatments on vegetable wash water, with a maximum microbial achieved by UV and O_3 treatments (Selma et al., 2008). The UV–ozone reactor is capable of removing most of the cyanide (66%) and of precipitating a significant amount of iron (46%) (Postigo and Richardson, 2014). The combined ozone–organic acid treatment was more efective than individual treatments in reducing initial population levels of Escherichia coli O157:H7 and Listeria monocytogenes pathogens on enoki mushroom (Yuk et al.,2007). The other study on atmospheric cold plasma device against Escherichia coli, Salmonella enterica Typhimurium and Listeria monocytogenes inoculated on cherry tomatoes and strawberries (Ziuzina et al., 2014). Resulted in reduction of E. coli, Salmonella and L. monocytogenes populations and also effectively reduced the background microflora of tomatoes. The antilisterial effects of coating formulation in combination with ozonated water, UV-C and C-ray treatments on inoculated samples ensured microbial safety during storage with a reduction of L. monocytogenes (Severino et al., 2014). Oxidation by ozone followed by sand filtration (SF) and powdered activated carbon (PAC) adsorption followed by either ultrafiltration (UF) or sand filtration. Despite its current higher costs and energy consumption PAC-UF treatment led to a good removal of most pollutants without forming problematic by-products, the strongest decrease in toxicity and a total disinfection of the effluent(Margot et al., 2013).As a sequential application, ozonation followed by electrolysis could be an optional process for the inactivation of some resistant microbial species, such as B. subtilis spore(Jung et al., 2013).

. Lastly, correlated UV, ozone and ionizing radiation methods by The ionizing radiation can be applied as an effective and economical alternative technique to conventional disinfection processes (Lee et al., 2015).

Conclusion

When exceeds a certain dose of ozone gas, damages to the environment and human health. Because of the harmful by-products, ozone is not recommended for indoor air cleaning. Hovewer, though significant reduction of bacteria and other contamination could be achieved by many of different emerging technologies when used alone or in combination, the disinfection of ozone appears to be successful administered at appropriate amounts and appropriate method even if many times they do not result in complete elimination.

Since the disinfection efficacy is greatly affected by the quality parameters of water itself, it is also important to understand the relation between the pH, temperature, turbidity and organic matter contents of substrates and the efficacy of disinfection.

Because of these reasons, discrepancies regarding the efficacy of ozone are often found in the bibliography and further research is still needed, to clarify and develop the inferences for its wider use, including the cost of its application.

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LANDSCAPE-ADAPTIVE APPROACHES TO THE MANAGEMENT OF SALINE SOILS FERTILITY IN UKRAINE

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Abstract

Saline soils of Ukraine are mainly formed in the Subboreal zone in the Forest-Steppe and Steppe natural zones. Conventionally they can be divided into two types - naturally saline and secondary saline. Secondary salinization is often an environmentally negative consequence of irrigation (during soil irrigation with mineralized water and during the rise of saline groundwater above the critical level). Naturally saline and secondary saline soils can be without morphologically indicated solonetzic horizon, their area is 1. 92 million ha and morphologically distinct solonetzic horizon (saline - alkaline) – 2. 8 million ha. Furthermore, among the irrigated lands 200 thousand ha of secondary saline of soils. The majority of saline soils in Ukraine are ploughed excluding strongly saline types and salt marshes. Fertility management of saline lands is aimed at building models of sustainable, environmentally safe and cost-effective land use. Effective use of saline soils in Ukraine is possible only with accounting soil properties (power and chemistry of salinity, salt balance of soil, etc.), the level of groundwater mineralization, climatic conditions (rainfall), the direction of agricultural use (arable land, hayfields, pastures, perennial plants). Depending on the genesis and properties of different types of saline soil for conditions of Ukraine, a landscape-adaptive set of measures to improve their fertility was developed. Herewith the reclamation is carried out in the following directions: chemical reclamation (soils and irrigation water), ameliorate plantation plowing, flushing, creating drainage systems, phytomelioration). Landscape-adaptive approach to the management of saline soil fertility ensures sustainable environmentally safe and cost-effective use of saline soils.

Keywords*:* *Saline soils, areas, properties, reclamation, fertility.*

Introduction

The general tendency of the agriculture development in the world at the present stage envisages the creation of conditions for the stabilization and increase volumes of agricultural production to solve the food problem. Year by year the Ukraine's role increases as an agricultural country in the food security of Europe and the world. According to the Land Registry of Ukraine, the land fund country makes 60.3 million hectares, of which agricultural lands – 41.6 million hectares (69% of the total area of Ukraine) in arable land - 35.5 million hectares (53. 9%) (Laktionova *et al*., 2015). Saline soils cover relatively small part of arable land area in Ukraine (about 7%), but require special attention in the management of agriculture for several reasons. Firstly, their agricultural development is closely linked to the need to perform a complex set of special reclamation measures for desalination. Secondly, soil salinity can occur during the exploitation of non-saline areas in irrigated agriculture as a result of the processes of secondary salinization. Thirdly, the range of saline soils is dynamic as soil salinity can be accompanied by the simultaneous appearance of the process of secondary salinization and alkalinization and new ranges of salinity on adjacent territories. Under the condition of landscape-adaptive scientifically substantiated use, saline soils are a significant reserve for increasing the production of various types of agricultural products.

Materials and Methods

Main research directions of saline soils in Ukraine: fixing manifestations and identification of patterns of sharing processes of salinization and alkalinization (depending on the structure of soil, ecological and reclamation land condition, irrigation water quality, existing farming technologies, etc.); spatial assessment of lands and directions for further development of the processes of salinization and alkalinization on the basis of their mapping and typing the territory; development of recommendations for the justification of measures to prevent or eliminate the salinity and alkalinity, protection and reproduction of soil fertility; formation of information support of management decisions systems in the context of monitoring and advisory - deliberative service (Baliuk *et al*., 2014).

The research was conducted in Forest-steppe and Steppe zones of Ukraine, where is disposed 98% saline soils. The objects of our research were:

naturally saline soils, irrigated soils and methods of its reclamation.

irrigation water. For irrigation in Ukraine are used basically water of main river arteries and created on their base water storage's and ponds.

agricultural plants, grown on saline soils in irrigating and none irrigating conditions (grains, vegetables, fodder's and technical cultures).

The main methods were field, model, analytical and statistical research.

Also were used

materials of large-scale land survey (1957-1961 yr.) and their correction;

the Land cadastre;

materials of agrochemical certification of the agricultural lands;

data of ecological-ameliorative monitoring;

data of scientific organizations National Academy of Agrarian Sciences of Ukraine, higher educational institutions and others.

For an accurate assessment of ecological and agromeliorative soil condition National Scientific Center «Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky» created a regulatory framework for the monitoring of saline soils, developed agro-environmental criteria, parameters and diagnostics of saline soils (using thermodynamic parameters - the activity of hydrogen ions, calcium, sodium, magnesium). To establish the degree of alkalinity, including the processes of secondary alkalinization, it is used a set of criteria: the amount of absorbed sodium and potassium cations, the ratio of the absorbed sodium and potassium cations to the sum of all cations, the ratio of the activity of sodium ions to the square root of the activity of calcium ions, as well as indicator of thermodynamic potential (pNa - 0.5pCa). The system of irrigation water quality assessment is developed and put into effect, which includes agronomic criteria (evaluation on salinity risk, alkalinization, alkalinity) and ecological criteria (soil pollution by heavy metals and fluorine), taking into account their buffer properties.

The control (monitoring) of the soils condition include:

- the systematic observation of state, properties, regimes of soils;
- the analysis of the stability of soils to the diverse degradation processes;
- the evaluation of the different types of the human economic activity, its positive and negative influence on the soil cover - impact-analysis;
- the computer data bases creation and cartography;
- forecast and prevention of the degradation processes.

The estimation of the soils condition is achieved by the method of comparison of the parameters of the soils, which are fixed in the initial period of observations, or standard soils with the same parameters after the corresponding periods of the soils using. The criteria of evaluation of the development of degradation processes are carried out on the basis of these observations, the levels of their ecological danger and unprofitability are determined (table 1).

Table 1. The integral estimation of the soil soils according to the degree of the degradation

Indices	Soil without degradation	Degree of degradation		
		Low	Average	Strong
Salinization, 0-50 cm				
Toxic salts content, eCl^-, meqv/100 g of soil	less than 0.3	0.3-1.5	1.5-3.5	more than 3.5
Ca:Na in water extract	more than 2.5	2.5-1.0	1.0-0.5	less than 0.5
Solonetzization, 0-30 cm				
Na^++K^+, % from sum of cations, clay soils	less than 3	3-6	6-10	more than 10
Na^++K^+, % from sum of cations, sandy soils	less than 5	5-8	8-12	more than 12
aNa/√aCa	less than 1	1-3	3-7	more than 7
Factor of dispersivity by Kachinsky, %	less than 10	10-20	20-30	more than 30
Alkalinization, 0-30 cm				
pH_w.	less than 7.8	7.8-8,5	8.5-9,0	more than 9.0
HCO_3^--Ca^{2+}, meqv/100 g of soil	less than 0.5	0.5-1.0	1.0-2.0	more than 2.0
CO_3^{2-}, meqv/100 g of soil	less than 0.1	0.1-0,3	0.3-0,9	more than 0.9
pH-pNa	less than 4.0	4.0-5.0	5.0-5.5	more than 5.5
Humus state, 0-50 cm				
Decreasing of humus content, % from initial	0	0-10	10-20	more than 20
Agrophysical state, 0-30 cm				
Content of air-dry aggregates 0,25-10 mm	more than 70	60-70	40-60	less than 40
Content of water-proof aggregates > 0,25 mm	more than 45	35-45	25-35	less than 25
Equilibrium density of composition, g/sm^3, clay soils	less than 1.3	1.3-1.4	1.4-1.6	more than 1.6
Equilibrium density of composition, g/sm^3, sandy soils	less than 1.3	1.3-1.5	1.5-1.7	more than 1.7
Pollution, 0-100 cm				
Heavy metals content, in zinc equivalents, mg/kg of soil	less than 25	25-50	50-100	more than 100
Water-soluble fluorine, mg/kg of soil	less than 6	6-10	10-20	more than 20

For today on the basis of the long-term comprehensive study of the soil processes dynamics and regimes in saline soils there are identified common landscape-zonal patterns and spatially differentiated features of orientation, and the prevalence rate of soil processes. For a more accurate spatial assessment of the saline soils state, a series of electronic maps of the regional and local levels is created.

At the regional level there are solved issues of spatial differentiation of land on the main factors of the formation and the degree of manifestation of salinity and alkalinity, the directions of further development of processes for determining a set of preventive and prophylactic measures. At the local level - the solution both monitoring tasks, and tasks related to the needs of the targeted land users. The main attention is paid to the definition of local variability of salinity and alkalinity manifestations, obtaining the characteristics of the soil condition and the dynamics of its performance. This approach allows creating a unified system of information and mapping support for measures to control environmental and agromeliorative state of naturally and secondary saline soils and solonetzic soils, developing the principles of their rational use based on technology adaptability to soil - climatic and social conditions.

Results and discussion

Naturally saline soils of Ukraine are confined to the two tectonic cavities - the Dneprovsko-Donetskaya (Forest-Steppe zone) and the Black Sea (Steppe), where the total lack of areas

drainage creates favourable conditions for the accumulation of salts (Novikova, 1984; 1996). (Fig.1).

Fig.1 Dissemination of saline soils in Ukraine

According to the soils classification of Ukrainian, they are presented by chernozems saline and solonetzic, chernozem meadow, meadow - chernozem, meadow-chestnut, meadow, alluvial - meadow, dark -brown and brown alkaline soils, solonetzes and salt marshes (Polupan *et al*., 2005).

Manifestation of secondary salinity is often the environmentally negative consequence of irrigation. Considering the fact that in Ukraine every two years from the five are arid, irrigated lands are the insurance fund of the country, as 75% of the territory of Ukraine for natural hydration are in unfavourable or partially favourable for agricultural production conditions, which greatly affects the efficiency.

Most often, secondary salinity in Ukraine occurs in conditions of inadequate drained low-lying areas by the absence of the collector-drainage network and / or by using for irrigation of saline water (more than 1 g / l) (Fig.2).

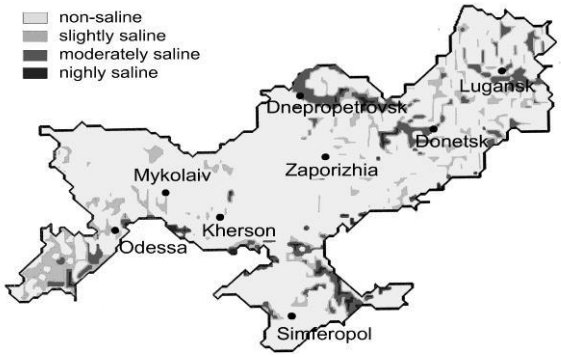

Fig.2 Dissemination of secondary salinity in Ukraine

Herewith it is set a positive salt balance in excess of accumulation of salts over their removal (Baliuk *et al*., 2009). The causes of secondary salinity in Ukraine can also be:

adverse chemical composition of the irrigation water in which the content of alkaline salts of sodium, potassium, in an equivalent ratio exceeds the content of salts of calcium, magnesium, iron and other two-and trivalent cations;

rise to the surface of the ground water with the same adverse chemical composition and so secondary salinity can be stored for a long time. Then, during salt flushing by precipitation or irrigation water in soils secondary alkalinization process can be developed;

special flushing of saline soils, as well as the development of the rice systems based on naturally saline soils (the early years).

According to the soils classification of Ukrainian, secondary saline soils are represented by chernozem ordinary, southern and dark chestnut soils (Polupan *et al*., 2005).
Until 1991 fertility improvement of saline soils in Ukraine was carried out mainly with the use of chemical amelioration on the area 2 million ha. After 1991, the reclamation of saline soils has been given insufficient attention mainly due to high energy consumption and the lack of adequate state financial support. During this period, the agriculture of Ukraine in general has gone on the way of extensive development, which inevitably leads to the loss of soil fertility, reduced yields and volatility of gross yield of agricultural products. This has contributed to changing attitudes, principles, methods and technological solutions to problems of land reclamation (Baliuk *et al*., 2015).
The feasibility of reclamation, its kind, technology are defined by agro-climatic resources, modern ecological and land improvement state of solonetzic soils, tasks of agricultural production and its resourcing. The main requirement for rational use of solonetzic soils in Ukraine in modern conditions should be considered a necessity of landscape-geochemical assessment of their formation and distribution and adaptive application of different types of reclamation. It provides protection and increase effective fertility of solonetzic soils, optimization of living conditions of the crops.
The State Agency of Ukraine for Land Resources has developed and approved a procedure for land conservation, based on which the saline soils containing toxic salts more than 0,4% are subject to transfer from agricultural lands to other land uses. The system of measures on re-naturalization of landscapes provides also removal of strongly solonetzic lands from arable land.
Chemical reclamation in Ukraine is recommended to carry out on saline and alkaline soils, which lend themselves well to this reclamation measure (chernozems saline and solonetzic, chernozem meadow, dark -brown and brown alkaline soils with share of solonetzes spot 10-30 % and 30-50 %, soils that irrigated by bonded suitable water). Continuous chemical reclamation is recommended to replace by the sample (contour) application of meliorant on solonetzes stains or by local application of gypsum in rows during sowing, providing economic- and material resources savings. According to this approach, areas of lands needing chemical reclamation could be significantly reduced compared to those that were reclaimed in previous years. This method of chemical reclamation provides increasing yields of grown crops at 15-20%. According to research, chemical reclamation should be repeated every 5-7 years. For the reclamation of secondly saline soils it is recommended to apply the gypsum directly into the soil or with irrigation water.
In the dry conditions of Steppe, where the chemical reclamation in unirrigated conditions is ineffective, it is recommended the use of reclamation plantage ploughing. It is expedient to apply for dark chestnut and chestnut saline soils in combination with steppe solonetzs (25%) with 40-50 cm deposition of carbonates and / or gypsum and alkali-saline brown soils in meadow-chestnut. With the research of the properties of agrotransformed solonetzic soils of Dry Steppe of Ukraine in irrigated and non-irrigated conditions, conducted by NSC ISSAR, was determined that the result of the aftereffect of reclamation plantage ploughing is the formation of highly environmentally sustainable agro transformed soils which are unique in nature on their morphological, agrophysical and physico-chemical properties and are able to

provide high productivity of agricultural crops, that are able to provide high fertility in agro-climatic conditions of the Steppe zone of Ukraine. A single conducting reclamation plantage ploughing provides a positive after-effect on soil properties and productivity of agricultural crops for 50-60 years and the discontinuation of its positive aftereffect is unmarked. Gain yields are 20-25% in unirrigated conditions and up to 40% under irrigation.

Large areas (92.2 thousand hectares) are occupied by solonetzic soils, where the reclamation is inefficient n and bringing them to arable lands is economically unfeasible. It is mainly saline alluvial chernozem and its complexes, with medium to high degree of salinity and the level of ground water occurrence of 1.0-1.5 m and alkali-saline chernozem soils and solonchaks. Cost-effective and environmentally safe using such soils is appropriate through selection of adapted economically valuable kinds of crops to create productive perennial multicomponent agrocenoses.

Saline soils with not deep mineralized groundwater with adequate natural drainage of areas are recommended for usage under rice systems. For reclamation of slightly saline soils it is recommended to use technical measures: application of organic and mineral fertilizers, the introduction of perennial grasses in crop rotation, phytomelioration.

To separate group it should be attributed saline soils, which in the present shortage of resource expenditure do not require radical reclamation. Increasing their fertility is advisable to make with farming practices and application of increased amounts of organic and mineral fertilizers, involvement in crop rotation perennial grasses and solonetz-stable crops. It is chernozems and dark chestnut soils with a low degree of salinization without solonets spots or their complexes with solonets spots 10%.

Conclusion

On the basis of generalization and systematization of long-term research there were highlighted the issues of the genesis of saline soils in Ukraine, the main directions of research, the main approaches to their rational use and fertility management. Landscape-adaptive approach to the management of saline soil fertility is proposed. The proposed system of reclamation activities, differentiated by features of different types and kinds of solonetzic soils, environmental and economic aspects of reclamation can reduce the area of chemical reclamation of solonetzic soils to 1.0-1.1 million hectares compared to 2.0 million hectares in previous years and receive economic benefit by increasing crop productivity and improving product quality. Thus obtained results will serve as for securing the sustainable development of agriculture in Ukraine.

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IMPACTS OF CLIMATE CHANGE AND ADAPTATION ON THE AVAILIBILITY OF WATER: A CASE OF AGRICULTURE IN PAKISTAN.

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Abstract

Climate change is a major issue faced by the world today. It is proved that activities by mankind are the main cause behind it. Among them Energy is the main activity that is responsible for most of the green house gases that cause climate change. Climate of a region is a key factor that ascertains the availability of water resource. The global phenomenon of climate change has increasingly instigated rise in average global air and temperature of oceans. Mounting global sea levels and pervasive melting of glaciers are evident consequences of climatic changes in the last few decades. Changes in atmospheric temperature and radiation balance are closely linked to the hydrological balance. The hydrological cycle gets intensified due to higher temperature of climate, which in turn results into increasing rate of evaporation and liquid precipitation. It is quite evident that these processes along with the changing pattern of precipitation have profound impact on the spatial and temporal distribution of soil moisture, groundwater reserves and runoff etc. This could trigger the intensity and regular occurrence of floods and droughts. Agricultural demand, particularly for irrigation water is considered more sensitive to climate change. A change in field-level climate may alter the need and timings of irrigation. The demand for water for irrigation purpose may increase with the increase in dryness and on other hand it may reduce if soil moisture content rises at critical times of the year. It is projected that most irrigated areas in Pakistan would require more water around 2030 and global net irrigation requirements would increase relative to the situation without climate change by 3.5–5% by 2030, and 6–8% by 2075. The effect of climate change on water resources may be mitigated through better water harvesting and by micro-storage facilities in watersheds. These would not only provide supplemental irrigation but also recharge the groundwater aquifers.

Key words: *Hydrological balance, radiation balance, liquid precipitation, atmospheric temperature, watersheds*

Introduction

Pakistan is one of the important South Asian countries with total geographical area of 796,095 km^2 and population of 19 million people that is 2.56% of the world population. The land area of Pakistan is 97.13% and water is 2.87%. Over 70% of Pakistan’s population is rural and agriculture oriented for whom these rivers are the source of their livelihood and prosperity.

The source of main water in Pakistan is canal irrigation system. The Indus valley, comprising the planes of Punjab and Sindh is mainly dependent on the water of river Indus and its tributaries, as the area is mostly arid because of annual precipitation. The river Indus is the life line of Pakistan's agriculture. Nearly 450,000 sq.m, Himalayan watershed of Indus and its tributaries includes the world's biggest glaciers outside the Polar Regions.

The distribution of rainfall in Pakistan varies on wide ranges, mostly associated with the monsoon winds and the western disturbances, but the rainfall does not occur throughout the year. Like, Khyber Pukhtonkhuwa (northern mountains) and Balochistan provinces receive maximum rainfall in the months of December to March while Punjab and Sindh receive 50 - 75% of rainfall during monsoon season (Kazi, S. A. and Khan, M. L 1951). The precipitation received in the country can be divided into two main seasons, summer or monsoon and winter

precipitation. The monsoon rainfall enters Pakistan from east and north east during the month of July to September. During this duration a good amount of rainfall is received in the north and northeastern areas of the country. Winter precipitation (December to March) are mainly received from western disturbances entering from Iran and Afghanistan. Table 1 depicts the major river basin system of Pakistan with their origin and catchment areas and average annual potential in river.

Table 1: Major river basins of Pakistan

River	Origin	Catchment area	Average annual potential in river (Bm3/yr)
Sutlej River	Western Tibet in the Kailas Mountain range and near the source of Rivers Indus, Ganges and Bramaputra	47,100 Sq.miles/75,369 Sq.km (70% in India)	45.67
Ravi River	Originates from the lesser Himalayas Range in India	15,600 Sq.miles/24,960Sq.km	65.87
Chenab River	Forms at the confluence of streams Bhaga & Chandra which join at a place called Tandi in Occupied Jammu & Kashmir state. -Upper most part is snow covered and forms the North East part of Himachal Pradesh -From Tandi to Akhnur the river traverses through high mountains	26,100 Sq.miles/41,760 Sq.km	34.59
Jhelum River	Originates in the Kashmir Valley about 34 miles (54 km) east of Anant Nag.	24,500Sq.miles/ 39,200Sq.km	58.76
Indus River	One of the largest rivers of the world and the main river of the Indus valley; -Originates near lake Mansarowar on north of Himalayas range in the mountain of Kailash Parbat in Tibet at an elevation of 18,000 ft.;	1,80,000 Sq.miles/2,88,000 Sq.km	647.45

The Indus River Basin is of major importance to the economy of Pakistan as well as other countries in South Asia. Indus River System consists of six main rivers (the Indus, Jhelum, Chenab, Ravi, Sutlej, and Kabul rivers) originating from glaciers in the Western Himalayas and provides irrigation to more than 16 million hectares of agricultural land and generates up to 13 giga Watts of electricity through hydropower plants in Pakistan, India, and Afghanistan. Glacial retreat and changes in precipitation patterns from anthropogenic climate changes are expected to alter significantly river basin behavior and jeopardize hydropower generation and irrigated agriculture production. Initial short-term increases in water flow may endanger the sustainability of downstream infrastructure. Expected long-term reduction in water flows will

reduce power generation potential and irrigation supply, with dramatic impacts on overall agriculture yields of these countries, consequently altering people's livelihoods. This will be particularly catastrophic for Pakistan where an estimated 50 % or more of river run-off flowing into the Indus Basin Irrigation river basins. Table 2 present the water resources and their composition in terms of availability and utilization as well as various end uses of sectors in the economy. It is quite evident that agriculture is the main sector which has major share of consumption of water for irrigation purpose.

Table 2: Water resources of Pakistan

Parameters	**Amount**
Annual precipitation	< 100 mm in parts of the Lower Indus Plain to > 750 mm near the foothills in the Upper Indus Plain
Utilizable water	169,384 billion m^3
Available water resources per capita	3,420 m^3
Surface water (storage and diversion)	147 MAF
Ground water (replenishible)	50 MAF
Present utilization (surface water 63%, ground water 37%)	176 MAF
Water use for irrigation (out of 169,384 billion m^3)	83%
Water use for domestic purposes	2%
Water use in industry, energy and other sectors	5%

Increase in persistency of drought requires a re-examination of our assumptions, operating norms and contingency measures for existing and planned water-management measures. Droughts produce the additional burden of migration of population from neighboring regions into lands that are already stretched to the maximum. Besides the social disruptions, there is the cost of the degradation of the natural resources due to the over-exploitation.

The United Nations report in 1997 determined each member country's ratio of water-consumption to water-availability, in order to gauge the overall pressure on its water-resources. The report predicts that moderate to high water-stress translates to consumption-levels that exceed 20 per cent of available supply. In Pakistan, this ratio has already exceeded 65 percent! Water-use in agriculture is projected to increase as our food-demand rises. World-wide, agriculture accounts for about 70 per cent of water-consumption; in Pakistan, its share is already 95%. Future projects show a 50- to 100-percent increase in demands for irrigation water by 2020. Figure 2 depicts the water requirements of the country in different sectors.

Water Requirements

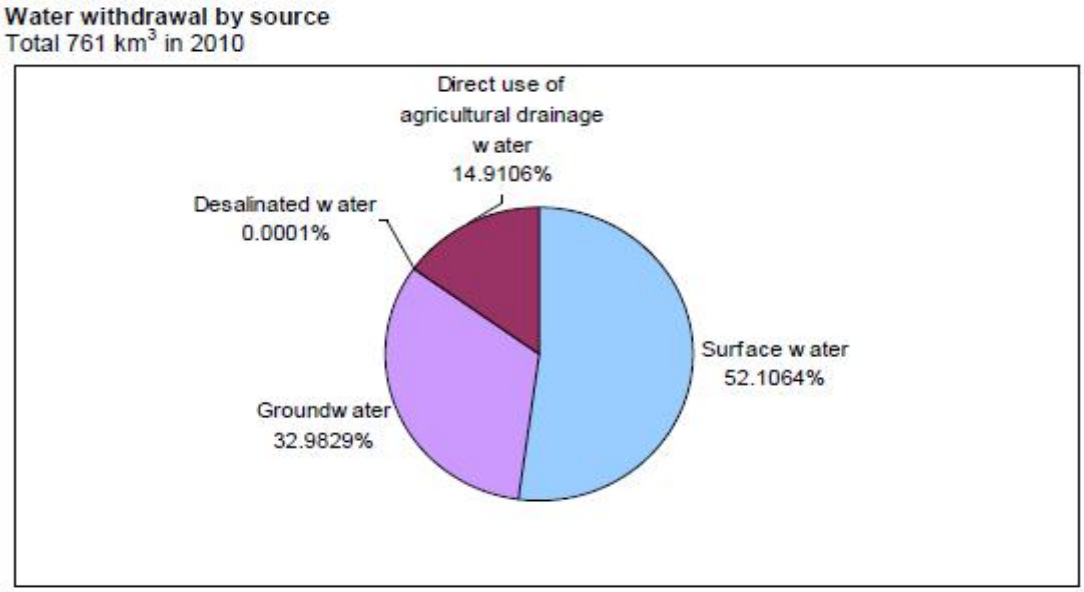

Fig 2: Water withdrawal by source

Agricultural Demands

Pakistan is a country, which is required to double its annual food production every 15 years, in order to maintain its statusquo in meeting requirements of food. This target, on the surface, may not look so demanding, as the country is bestowed with enough fertile and productive lands and sufficient freshwater-resources. Despite the availability of these basic resources, unfortunately the country has to import large quantities of food commodities every year. With the current population of about 140 million people growing at the rate of almost 2.5% per annum, the country would have to feed 120 million additional mouths by the year 2025. Table-3 shows the production and water-requirements of some major crops needed to maintain self-sufficiency in these food grains, which may be compared with future water needs.

Table 3: Agriculture Water Demands (MAF)

Crops	**1990**	**2000**	**2025**
Wheat	26.27	28.8	56.91
Rice	18.78	22.24	16.68
Cotton	13.68	15.71	19.35
Sugarcane	11.35	13.41	13.93
Other Crops	28.93	30.59	46.74
Total with Losses @70%	168.32	188.28	261.14

Domestic and Industrial Needs

Table - 4 shows the domestic present and future domestic requirements, based on a per-capita demand of 46m3 per annum. The corresponding industrial water-demand is considered negligible when compared with the domestic and agricultural demands.

Table 4: Water Demand for Domestic Use

Year	**1990**	**2000**	**2025**
Population (Million)	110	140	260
Water Demand (MAF)	4.1	5.2	9.7

Pakistan is highly vulnerable to climate change as its economy is heavily reliant on climate-sensitive sectors like agriculture and forestry, and its low-lying densely populated deltas are threatened by a potential risk of flooding. In Pakistan, annual mean surface temperature has a consistent rising trend since the beginning of 20th century. Considering Table 5, rise in mean temp. of 0.6-1.0°C in arid coastal areas, arid mountains and hyper arid plains, 10-15% decrease in both winter and summer rainfall in coastal belt and hyper arid plains, 18-32% increase in rainfall in monsoon zone especially the sub-humid and humid areas is observed. There is 5% decrease in relative humidity in Balochistan, 0.5 to 0.7% increases in solar radiation over southern half of country.

Table 5: Temperature Change during 1951 – 2000

S.No	**Region**	**Trend during last century**	**Range (°C)**
1	Over the Thermal Low Region	(Increased)	0.2 to 1.0°C
2	Coastal Areas (Balochistan)	(Decreased)	0.5 to -1.5°C
3	Monsoon belt	(Generally decreased)	2.0 to 0.0°C
4	Northern Mountains (Greater Himalayas)	(Generally increased)	1.5 to 1.5°C
5	Thar region	(Increased)	0.3 to 1.0°c
6	Sindh Coast	(Generally Increased)	0.0 to 0.5°c

There is 3-5% decrease in cloud cover in central Pakistan with increase in sunshine hours, 3-5% increases in ETo due to 0.9°C temperature increase. 5% Increase in net irrigation water requirement with no change in rainfall. Expanding aridity in Northern parts outside monsoon range and arid regions. During last 100 years, 7 strong, 10 moderate and 7 weak EL-Nino events 17-64% departure of rainfall from normal during strong events.

Projected Changes in Precipitation and Temperature

SCHENGEN model was used for generating climate change scenarios during next half of 21st century in Pakistan, based on 1931-2010 data. Results indicate a progressive change in temperature during the period. However changes in rainfall pattern may not be uniform and south-western coastline and western Balochistan may experience low rains.Results indicate that during monsoon, temperature change over seasonal low is relatively slow compared to other arid and hyper arid region due to windy conditions?

Potential Impacts of Climate Change

Global weather changes and water- resources are deeply inter-related. The largest source of freshwater is rain. Global climatic changes will have major effects on precipitation and runoff. In the relatively arid and semi-arid regions, modest changes in precipitation can have proportionally large impacts on water supplies. In mountainous watersheds, higher temperatures will increase the ratio of rain to snow, accelerate the rate of spring snowmelt, and shorten the overall snowfall season, leading to more rapid, earlier, and greater spring runoff. Because the temperature projections of climate models are less speculative than the projections of precipitation, temperature-induced shifts in the relative amounts of rain and snow and in the timing of snowmelt in mountainous areas are considered highly likely. Climate-induced changes in hydrology will affect the magnitude, frequency, and costs of extreme events, which produce the greatest economic and social costs to humans. Flooding could become more common and extreme. Recent reports of the Intergovernmental Panel on Climate Change (IPCC) suggest that a greenhouse warming is likely to increase the number of intense- precipitation days as well as flood-frequencies in northern latitudes and snowmelt-driven basins. These reports also suggest that the frequency and severity of droughts could increase in some areas, as a result of a decrease in total rainfall and more frequent dry spells.

According to the climate change scenario, the global warming should be reflected in the river-flow data of Pakistan, especially in the nineties. From the data available from 1975 to 2000, the following picture emerges. In the post-Tarbela era, the water diverted from the rivers to the canal system has remained constant around 104 MAF per year (128 bm).
The fresh-water outflow to the sea may, therefore, be considered to be a good indication of the total river flow for this period. From the year 1975/76-1989/90 the average flow into the sea per year has been 34.13 MAF/year. However from the year 1990/91 to 1999/2000, this flow has been 47.88 MAF/year i.e. about 40 % greater. For the winter season for the same period, the average flow into the sea from the year 1975/76-1989/90 was 1.86 MAF/year. However from the year 1990/91 to 1999/2000, this flow has been 3.28 MAF/year, i.e. about 76 % greater. Although other factors may also be contributing, nevertheless the change visible in this time-frame is quite large. This increased river-flow could be taken as evidence for local effect of global warming. The global warming impact is expected to impact initially on the river-flow to increase, as the glaciers melt, then decrease as they recede.

Diminishing flows in Indus Basin

Glacial melt water is a key source of water for river system in Pakistan. However, along the eastern slopes of the Himalaya, glacier cover has decreased rapidly in recent years, and total cover is now approaching the lowest experienced in the past 10 000 years.

The Gangorti glacier is retreating 98 feet per year. At this rate scientists predict the loss of all central and eastern Himalayan glaciers by 2035. As the glacial cover has decreased, so have the downstream flow volumes. Analysis of precipitation and inflow data shows a direct relationship indicating that, in dry years inflows are reduced despite the fact that temperatures were higher in upper watersheds. This finding appears to contradict projections of the Intergovernmental Panel on Climate Change that warmer temperatures will cause glacial contributions to downstream flow regimes to increase in the short term. However, historical stream flow data indicates that this increased flow phase has already passed, and that the basins have entered a potentially long-term trend of declining flows. The continuation of this trend would exacerbate water shortages that were already apparent across the country during recent severe drought. Increased temperature with or without any change in precipitation, over

the last few decades is causing glacier melting leading to higher rates of sliding and sediment loads in the upper watershed.

Quality of Fresh Water

Water quality would suffer from the projected impacts of climate change. Poor water quality effectively diminishes the availability of potable water, and increases the costs associated with rendering water suitable for use. Changes in water quantity and water quality are inextricably linked. Lower water levels tend to lead to higher pollutant concentrations, whereas high flow events and flooding increase turbidity and the flushing of contaminants into the water system.

Conclusion

The developing countries of Asia, like Pakistan, where impacts of climate change are likely to be felt most severely because of resource and infrastructure constraints, need to develop and implement incremental adaptation strategies and policies to exploit no regret measures and stressing the importance of considering climate change in planning, designing and implementing development activities.

The first is a macro strategy and involves rapid sustainable and equitable development that will increase income levels; education and technical skills; improve public food distribution, disaster preparedness and management and health care systems and reduce vulnerability. The second strategy is a micro strategy and involves the management of sectors most sensitive to the climate change. This means developing new institutions or modifying existing ones to promote adaptation to climate change. It would also involve modifying climate-sensitive infrastructures already planned or implemented or other long-term decisions that are sensitive to climate.

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ROLE OF PREDATORY MAMMALS IN CIRCULATION OF ZOONOTIC HELMINTHS IN THE URBAN AREAS

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Abstract

Work contains materials on helminthes, parasitizing at predatory mammals in the conditions of the urbanized territories of the Voronezh region. In the studied territory at predatory 33 helminthes species is revealed. Among them nematodes (17 species) dominate, further follow cestodes (9 species) and trematode (6 species), the minimum number of species is noted among acanthocephalan (1 species). In the urbanized territories, more than 20 species of activators zoonotic helminthosen circulate. Results of these researches show that the red fox plays important role in maintenance of functional stability of the natural centers zoonotic helminthosen. In the urbanized territories, the red fox actively joins in circulation zoonotic helminthosen.

Keywords: urbanized territories, zoonotic, predatory mammals, helminthosen

Introduction

Circulation helminthosen is represented as difficult structured process caused by a combination of various ecology-biological factors. A specific variety and number of local populations belong to these factors helminthes, secondly, a biodiversity of animals owners, thirdly, structure and quantitative parameters of biotic and abiotic components in ecosystems, first. Interaction of the listed factors influences features and regularities of realization of life cycles and functional stability of helminthes parasitic systems, respectively, circulation helminthosen (Galaktionov, Dobrovolskij, 2003; Roitman, Beer, 2008).

Now considerable influence on shape and a condition of ecosystems renders anthropogenous factors, which can change the qualitative and quantitative status of certain species of parasitic worms. Due to the features of ecology helminthes, three main forms of circulation helminthosen are allocated: natural, anthropogenous and natural and anthropogenous. Natural forms are dated for natural ecosystems where circulate helminthes in populations of wild animals. Parasitic worms are considered as important components of natural ecosystems which influence an organism, populations and communities of animals hosts (Hechinger et al., 2008). Anthropogenous forms are connected with the urbanized territories where the leading role in circulation helminthes is played by pets and the person. The natural and anthropogenic form arises in adjacent (buffer) territories of natural and anthropic ecosystems. These conditions influence formation of a certain biodiversity helminthes and specialized ecological communications. There is a system of optimum factors for transfer of activators helminthosen on the urbanized territories and their subsequent adaptation in a format of anthropogenic circulation (Romashov, 2009).

In circulation of numerous group zoonozny гельминтозов wild predatory mammals have importance. Predatory (Carnivora) - one of the most representative on a specific variety and number of groups of animals among mammals. They occupy the highest levels of an ecological pyramid and have wide tropology-horology communications. At predators the numerous fauna on a specific variety helminthes as a part of which the considerable share is presented by activators zoonotic helminthosen was created. Predatory mammals as the integral components are built in process of circulation of activators of the majority natural

centre helminthosen and play a role of obligate owners and reservoir (Pozio et al., 2001; Deplazes et al., 2004; Romashov et al., 2006; Denzin el al., 2009).

Taking into account ecological and ethological factors between wild and house predators there are various trophic communications which cause an active exchange helminthes. House predatory (dogs and cats) are carriers of a large number helminthes and important links in ecological chains of circulation zoonotic helminthosen (Dyachenko V. et al., 2008). It is established that with participation of wild and house predatory more than 70% zoonotic helminthosen circulate.

Now concerning considerable number zoonotic helminthosen strengthening of epizootic intensity in connection with big number of vagrant dogs and cats in the cities and in rural settlements is registered. On the other hand separate species of wild predators, for example, a fox and a wolf, possess high ecological plasticity. These types successfully adapt in anthropogenic conditions and actively participate in circulation zoonotic helminthosen. In this regard. researches on fauna and ecology studying helminthes in the conditions of the urbanized territories have scientific and practical value and are directed on the solution of tasks in the field of applied medicine, veterinary science and ecology.

Results of research are given in the real work helminth fauna and ecological aspects of circulation zoonotic helminthosen in the conditions of the central part of the European Russia (Voronezh region). The quantitative analysis of fauna and contamination of predatory mammals of helminthes, with an assessment of their number and occurrence is carried out.

Materials and methods

Original materials of helminthes are collected from predatory mammals in various regions of the Voronezh region on an extent more than the 20-year period (1990-2014). The method of complete helminthological openings (Ivashkin et al., 1971) investigated over 200 individuals of the predators relating to 12 types: the canidae - 4 species (a wolf, a fox, a raccoon dog, a house dog), marten – 7 species (a badger, an otter, a wood marten, a stone marten, steppe polecat, the american mink, caress), cat's - 1 species (a house cat). Materials are received from animals at carrying out regulation actions, registration researches and from victims of animals. Quantitative indices of contamination and distribution helminthes in owners estimated on the following indicators: occurrence (extensive of infection), intensity of an infection, abundance index (Beklemishev, 1970).

Results and discussion

The received helminthological materials are analyses in the following directions. First, the modern helminth fauna wild and house carnivorous is investigated quantitative data on contamination helminthes owners are secondly obtained the differentiated assessment of quantitative parameters concerning epidemiologically significant species helminthes, circulating with participation wild and house predators is thirdly made.

By results of researches at wild predators 33 helminthes species, presented by four taxonomic groups - Trematoda, by Cestoda, Nematoda and Acanthocephala is revealed (tab. 1). The maximum number of types is noted as a part of nematodes (17 species), further follow cestodes (9) and trematodes (6), the minimum number of species is revealed among acanthocephala (1). Among predators the highest indicators of a helminthes species variety are noted at a fox - 24 species (tab. 1). At other species of predators a helminthes species variety is essential below: at a wolf it is revealed - 12 species, a stone marten - 11 species, a raccoon dog and a wood marten - on 9 species, the american mink - 8 species, a badger - 7 species. The minimum number of helminthes species is revealed at an otter - 3, caresses - 3, steppe polecat - 1.

Table 1 Helminth fauna at wild predators in the territory of the Voronezh region

The helminthes species	The host species									
	wolf	fox	raccoon dog	badger	wood marten	american mink	badger	otter	steppe polecat	weasel
Trematoda										
Alaria alata	+	+	+	+			+			
Opisthorchis felineus		+	+			+		+		
Pseudamphistomum truncatum		+	+			+		+		
Metorchis bilis		+				+		+		
Euparyphium melis			+			+	+			+
Mamorchipedum isostoma						+				
Cestoda										
Taenia hydatigena	+	+								
T. pisiformis		+								
T. martis				+	+					
T. crassiceps		+								
T. krabbei	+	+								
Hydatigera taeniaeformis		+								
Mesocestoides lineatus		+			+					
Dypilidium caninum	+	+								
Echinococcus multilocularis		+								
Nematoda										
Toxocara canis	+	+								
T. mystax		+								
Toxaskaris leonina		+								
Askaris columnaris						+				
Uncinaria stenocephala	+	+	+	+	+		+			+
Strongyloides martis					+				+	
Dirofilaria immitis		+								
Crenosoma vulpis	+	+			+					
Capillaria putorii		+	+	+	+	+	+			+
C. plica	+	+	+							
C. mucronata				+	+	+	+			
C. hepatica	+									
Eucoleus aerophilus	+	+	+	+	+		+			
E. boehmi	+	+								
E. trophymenkovi				+	+					
E. paranalis				+	+					
Trichinella nativa	+	+	+	+	+		+			
Acanthocephala										
Macracanthorhyn-chus catulinus		+								

The leading role in circulation zoonotic helminthosen is played by a fox of which share the maximum number of species in structure helminth fauna predators is the share. Quantitative parameters of occurrence and number helminthes, parasitizing at a fox are analyses, the measure of representation of separate species helminthes is inadequate (tab. 2). Taking into account occurrence indicators (extensiveness of an infection) and an abundance (number) among helminthes four groups are allocated: dominants, subdominants, intermediate and rare (casual) species.

Table 2. Distribution helminthes at a fox on extent of domination

Helminthescategory	Helminthes species	Contaminationindicators	
		Extensiv of infection%	Index abundances (number), specimen
Dominants	*A. alata*	75,0	82,0
	T. canis	71,4	8,2
	E. aerophilus	62,5	4,3
	C. plica	53,6	3,25
Subdominants	*T. leonina*	34,0	2,2
	T. nativa	33,9	18,4 (in 1 g of muscles)
	T. crassiceps	33,3	3,2
	C. vulpis	25,0	3,7
	T. hydatigena	25,0	3,62
	U. stenocephala	23,2	2,5
Intermediate	*D. caninum*	17,8	3,9
	T. mystax	17,8	0,46
	M. lineatus	12,5	5,5
	O. felineus	11,1	0,5
	P. truncatum	11,1	0,4
	C. putorii	8,9	0,48
	H. taeniaformis	5,4	0,07
	E. boehmi	3,6	0,09
Casual	*T. pisiformis*	1,8	0,26
	T. krabbei	1,8	0,2
	E. multilocularis	1,8	0,2
	D. immitis	1,8	0,09
	M. catulinus	1,8	0,07
	M. bilis	1,1	0,8

The numerous helminth fauna at a fox is caused, in our opinion, by two major ecological factors, first, high number of this predator, secondly, its wide ecological valence. If to estimate a contribution of separate species of predators to formation process of helminth fauna and at maintenance of its «status quo», the leading role in this process belongs to a fox. Among registered helminthes (tab. 1, 2) a considerable part have epidemiological and epizootological value. In turn it assumes an active exchange helminthes with other types both wild, and the house carnivorous.

Let's note that in the territory of the Voronezh region, the fox, among predatory mammals, is the most numerous both in natural ecosystems, and on recreational and farmlands. In this regard the fox plays important role in maintenance of functional stability and circulation of a large number natural center zoonotic helminthosen. In this regard we analyses the original helminthological materials characterizing ecology-epizootology aspects circulating zoonotic helminthosen where the fox is an active and important link of this process.

By consideration of these materials the main accent is made on activators zoonotic helminthosen which taking into account literary data and own researches are registered as at pets, first of all dogs and cats, and at the person. Besides, across the Voronezh region there are

not numerous materials on fauna and distribution helminthes among the house carnivorous. At present for the studied territory from among zoonotic helminth species, found in a fox, it is necessary to consider the most part as "potential" activators zoonotic helminthes.
By results of the real researches at a fox 22 species of agent zoonotic helminthosen as a part of which each of taxon (at level of classes) is presented by various quantity of species are registered. The greatest number of species is revealed among nematodes - 10 species: *Toxocara canis, T. mystax, Toxaskaris leonina, Trichinella nativa, Dirofilaria immitis, Uncinaria stenocephala, Capillaria putorii, C. plica, Eucoleus aerophilus, Crenosoma vulpis* (fig. 1).

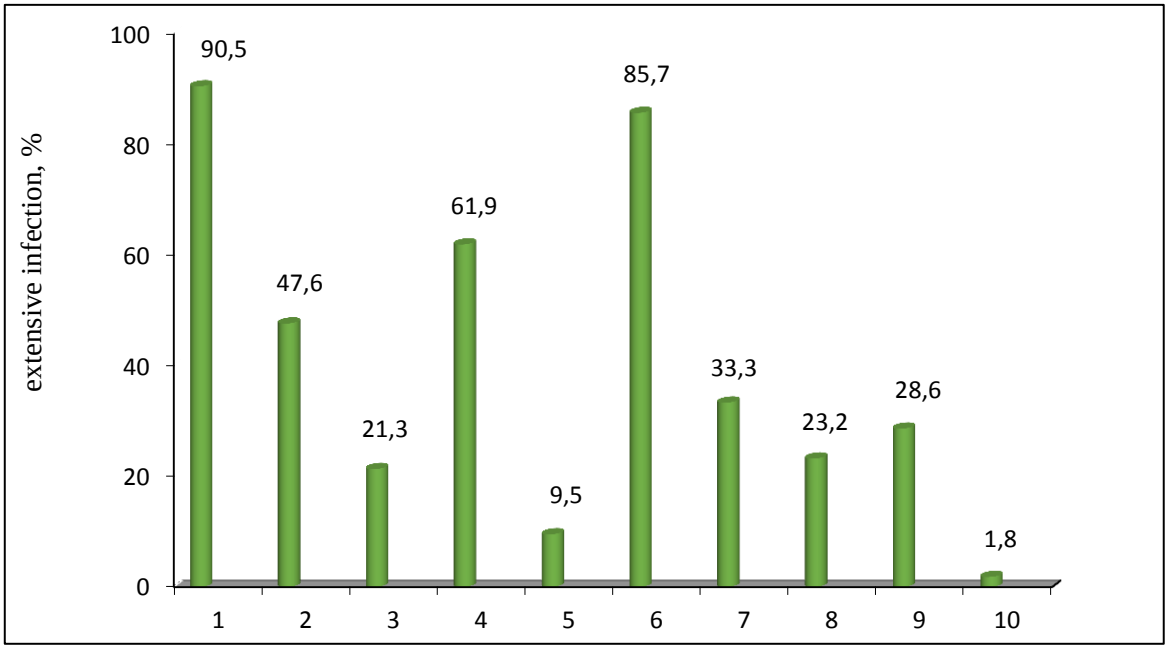

Fig. 1. Contamination of fox (extensive infection) nematodes (agent zoonotic helminthosen) in the urbanized territories of the Voronezh region:
1-Toxocara canis, 2 - T. mystax, 3 – Toxaskaris leonina, 4 - Capillaria plica, 5 - C. putorii, 6 – Eucoleus aerophilus, 7 – Trichinella nativa, 8 – Uncinaria stenocephala, 9 – Crenosoma vulpis, 10 – Dirofilaria immitis.

The highest index of extensive infection are noted at 4 species: *T. canis* (90,5 %), *E. aerophilus* (85,7 %), *C. plica* (61,9 %) and *T. mystax* (47,6 %). The second level is occupied by 3 species of nematodes, contamination with which hesitates from 20 to 30 %, including *T. nativa* (33,3 %). For other 2species of nematodes index of extensive infection less than 10 % are noted (fig. 1).
From among the registered nematodes from the point of view of applied medicine and veterinary science in the territory researches nematodes of the genus *Trichinella*, *Toxocara*, *Toxaskaris*, *Dirofilaria* and *Eucoleus* are the most actual. Now study of epidemiological and epizootological intensity in the relation nematodosis, which agent are nematodes of this group is observed. In particular, over the last 10 years trichinellosis and dirofilariosis got the status endemically circulating natural очаговых invasions in the territory of the Voronezh region (Romashov et al., 2006; Volgina et al., 2013).
By rather numerous group of number zoonotic helminthosen are presented trematodes - 4 species and cestodes - 8 species. Among trematodes registration at a fox of three opisthorchidae species (*Opisthorchis felineus, Pseudamphistomum truncatum* and *Metorchis*

bilis) (fig. 2) is remarkable. These facts indicate active participation of a fox in circulation of the natural centers opisthorchosis.

From number трематод the highest index of extensive infection and index of abundance are noted at *Alaria alata* (75,2 % and 82,0 specimen) (tab. 2, fig. 2). Now the increase in a hostal component of this trematode is observed, the range of definitive hosts, which actively include also the house carnivorous extends.

Contamination cestodes varies, including the highest indicators (from 25 to 30 %) are revealed at two species: *Taenia hydatigena* and *Taenia crassiceps*, *Mesocestoides lineatus*, the second level (from 10 to 20 %) occupy three look: *Dypilidium caninum*, *Mesocestoides lineatus* and *Hydatigera taeniaformis*, the minimum indicators are noted at *Echinococcus multilocularis*, *Taenia pisiformis* and *T. krabbei* (fig. 2).

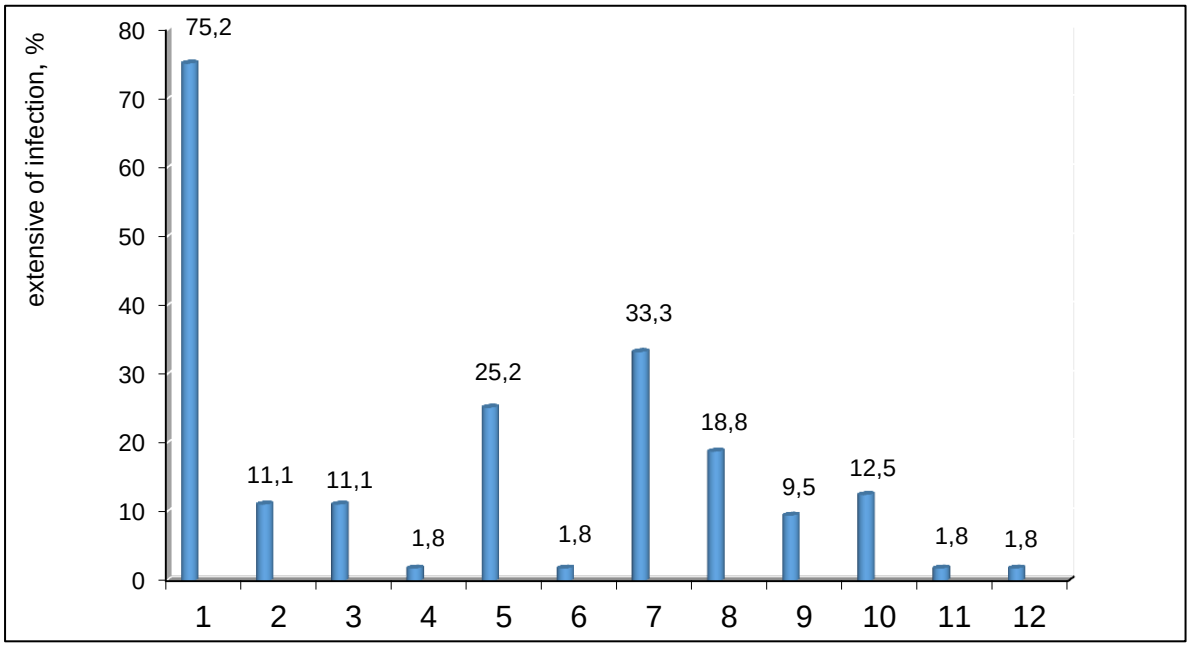

Fig. 3. Contamination of fox (extensive of infection) trematodes and cestodes (zoonotic helminthosen) in the urbanized territories of the Voronezh region: *1-Alaria alata, 2 – Opisthorchis felineus, 3 – Pseudamphistomum truncatum, 4 – Metorchis bilis, 5 – Taenia hydatigena, 6 - T. pisiformis, 7 - T. crassiceps, 8 – Dypilidium caninum, 9 – Hydatigera taeniaformis, 10 – Mesocestoides lineatus, 11 – Echinococcus multilocularis, 12 – Taenia krabbei.*

Results of these researches show that the fox plays important role in maintenance of functional stability of the natural centers and circulation of a large number of zoonotic infection. On the other hand in the conditions of anthropogenic ecosystems, for example, farmlands and rural settlements, the fox actively joins in circulation zoonotic helminthosen. In these conditions the anthropogenic component presented helminthozoonosen, which are dissemination mainly among housedogs and cats, is dominance. The fox in this case can play a role of an additional ecological link, as a source and a factor of accumulation and distribution of activators of these infections in the conditions of urbanized territories.

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CHARACTERISTICS OF DISTRIC CAMBISOL ON DIFFERENT PARENT SUBSTRATES IN THE AREA OF VLASENICA, BOSNIA AND HERZEGOVINA

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Abstract

Distric cambisols are very abundant in the Vlasenica area which is located in the eastern part of Bosnia and Herzegovina (BiH), occupying ¾ of the municipality's areas. They were formed on different parent substrates where the different properties of soil are inherited. The characteristics of dystric cambisols formed on sandstones and shale, and under different types of land use: arable land - maize, orchards - plum and meadows, were analyzed in this paper. The aim was to determine the influence of land use and management practices on the basic parameters of dystric cambisols on various substrates. The following physical properties were also analyzed in this paper: texture, real specific weight and bulk density, porosity, water retention capacity, permeability to water and air capacity. The chemical characteristics were analyzed as follows: active and substitution acidity of soil, humus and nitrogen content, availability of phosphorus and potassium. Profiles are open one per each land use, both on the sandstones (profiles 2, 3 and 8) and on shale (profiles 4, 10 and 11) parent substrates. The most favorable physical and water-physical properties were observed in land under plum orchards that are covered with grass and meadows on both substrates. The highest content of humus on shale in the Ah soil horizon was also recorded under arable land, and on sandstones in the Ah soil horizon under orchard. In the surface horizon in the soil under maize in both examined parent materials, the highest content of available phosphorus and potassium is recorded in comparison to the other tested variants of land use.

Keywords: Distric cambisol, land use, the parent substrate, the physical and chemical properties.

Introduction

Distric cambisols occupy a substantial part of the territory of BiH (28.73% of the area is distric cambisole which is formed on different parent material of the total land area in BiH), including the Vlasenica area, with 3/4 of the entire surface.

Parent material of substrate has a twofold impact on the process of soil formation with both physical properties of rocks (compactness, binding, looseness, the degree of dispersion) and mineral composition of rocks (impact on soil fertility), which has been confirmed in many scientific papers (Szafranek and Sklodowski, 2006; Knezevic et al., 2011).

Soil quality is significantly dependent on its natural origin and conditions of their origin, but it also depends on changes caused by its use (Kastori and Milosevic, 2011).

The aim of this study is to determine the impact of land use and management practices on the basic parameters of distric cambisols on various substrates.

Material and method

A total of 6 of the profile, the profile 3 on sandstones (sections 2, 3 and 8) and the profile 3 on the shales (sections 4, 10, 11) were analyzed. Profiles are open in the soil under different modes of use: arable land - maize, orchards - plum and meadows.

Area under meadows was previously used for growing field crops, 7-10 years ago. Manure is introduced to the land under maize every three years in the fall, and the fertilizer is used as a starter fertilizer and as a top dressing (urea, KAN). Plowing of crop residues is not performed. On the land that is under orchard, 5 years old, previous culture was a meadow (sections 8 and 4). In the row, and between the rows of orchard, land is under grass cover.

For laboratory analyzes, the soil samples in disturbed and undisturbed condition of all genetic horizons were collected.

Laboratory analyzes of soil were performed as follows:

Texture of soil is determined by the modified pipettes B method where the coarse sand is separated in sieve. Sample preparation for analysis was performed with Na pyrophosphate by Thun, and the texture class based on the classification by Ehwald.

Albert - Boggs's method was used to determine real specific mass of soil (S_{mp}, g/cm^3) with xylol, and the bulk density of soil (S_{mv}, g/cm^3) in the Kopecky´s cylinders of 100 cm^3 volume, by moistening to the retention capacity, weighing, drying at 105°C and re-weighing.

Total porosity (%) is determined by calculation of the difference between values of real and bulk specific soil mass ($P_{vol\%}=S_{mp} - S_{mv}/S_{mp}$ x 100).

According to the Kopecky method water retention capacity (% volume) was determined. Filtration coefficient (K-Darcy) is defined in the natural undisturbed soil samples (in the cylinders by Kopecky).

Air soil capacity is determined by calculation of the difference between the value of total porosity and water retention capacity.

pH value in H_2O and KCl were determined in a suspension of 1:2.5 on the pH meter electrometrically, and humus content was determined by Tjurin method in Simakov modifications in the calorimeter.

The total nitrogen content was determined by the Kjeldahl method in modification by Bremner. According to the Al method by Egner–Riehm–Domingo was determined content of available phosphorus and potassium

Results and discussion

Textural composition of soil

The fraction of silt is in average dominant in the Ah horizon profile of the sandstones and shales (Table 1).

In (B) horizon of the profile on the sandstones slightly higher average content of the sand fraction was recorded (40,18%) compared to the fraction of the silt (37,46%), while in (B) horizon of the profile on shales showed highest average content of clay (43,02%).

On both analyzed parent substrates, content of silt decreases with depth, while the clay fraction content increases.

The content of sand fraction increases with profile depth in the sandstones, and in shales profiles it decreases with the depth of the profile.

Table 1. Texture composition of soil

Parent substrates	Profile	Land use.	Sand (%)		Silt (%)		Clay (%)		Texture class (classification by Ehwald)	
			A_h	(B)	A_h	(B)	A_h	(B)	A_h	(B)
Sandstones	2	meadows	33,20	30,99	52,65	44,72	14,15	24,29	PI	I
	3	maize	38,08	39,69	43,28	37,37	18,64	22,94	I	I
	8	orchards	43,46	49,85	39,70	30,29	16,84	19,86	I	I
Average value			38,25	40,18	45,21	37,46	16,54	22,36		
Shale	4	orchards	30,37	25,75	41,19	39,20	28,44	34,83	I	GI
	10	meadows	18,76	7,91	50,05	40,11	31,19	51,98	P G	G
	11	maize	41,67	39,59	37,71	18,16	20,62	42,25	I	G
Average value			30,27	24,42	42,98	32,49	26,75	43,02		

I-loam; PI-silty loam; PGI-silty clay loam; GI-clay loam; G-clay;

Physical properties

Physical properties of dystric cambisols are mainly related to the parent substrate. The higher value of bulk density in the sandstones profiles compared to the shales profile is a consequence of different mechanical composition that is inherited from the parent material (Table 2).

Because of the large amount of humus, higher aggregation and porosity and lower subsidence of Ah horizon compared to deeper horizon, the value of bulk density in the analyzed profiles increases with depth. The maximum value of bulk density in the Ah horizon in the profiles of the sandstones and is observed in the profiles under maize. Increased bulk density in the soil which is cultivated in relation to the uncultivated is the result of continuous tillage (Idowu, 2005).

Real specific weight in both horizons profiles on sandstone and shales are slightly different in average.

The soil is porous in all samples, except in the (B) horizon of the profile 3 (maize) where low porosity is recorded. Somewhat higher average value of total porosity was recorded in both the horizons of the profiles in relation to the profiles on sandstones. Due to the increased presence of organic matter in the Ah horizon, total porosity in all tested profiles is higher in Ah horizon in relation to the horizon (B) (Tilahun, 2007).

Profiles under maize and shales in the Ah horizon have the lowest porosity. The tillage increases compactness within the arable layer and destroys most of the secondary pores; therefore, the lowest total porosity in the test soil was observed in the soil under maize.

The maximum porosity in the profiles on sandstones and shales in the Ah horizon was recorded in sections 3 and 11 under orchard. In all the tested samples, mean water retention capacity was recorded, except in both horizons of the profile 10 (meadow) and (B) horizon of the profile 11 (maize), where high water retention capacity was recorded.

There is s minor difference in average water retention capacity of the profiles of the sandstones and shales, while cambic horizon has high water retention capacity due to greater presence of clay fraction, the average value of retention of water capacity profile in shales is higher compared to average value water capacity retention of in the profiles of the sandstones.The highest retention of water capacity value in Ah horizon profile of the sandstones was recorded in the profile 8 under orchard, and profiles on shales in the profile 10 under meadow.

In Ah horizon of the analyzed profiles the soil is very permeable, and in (B) horizon the permeability is medium. The average permeability coefficient of Ah horizon in the sandstones and shales profiles and are slightly different, while in (B) horizon the permeability is greater in sandstone profiles than in shales profiles.

Table 2. Water-physical properties of soil

Parent substrates	Profile	Land use.	Bulk density (g/cm^3)		Real specific weight (g/cm^3)		Total porosity (%)		Water retention capacity (vol. %)		Filtration coefficient (m/day)		Air capacity (vol. %)	
			A_h	(B)	A_h	(B)	A_h	$(B)_h$	A_h	(B)	A_h	(B)	A_h	(B)
Sandstones	2	meadows	1,17	1,35	2,60	2,73	55,00	50,55	41,00	39,00	1,42	0,82	14,00	11,5
	3	maize	1,25	1,59	2,65	2,74	52,83	41,97	42,50	36,00	1,45	0,78	10,33	5,97
	8	orchards	1,13	1,49	2,64	2,79	57,20	46,59	44,50	36,00	1,20	0,49	12,70	10,5
Average value			1,18	1,48	2,63	2,75	55,01	46,37	42,67	37,00	1,36	0,70	12,34	9,37
Shale	4	orchards	1,05	1,40	2,62	2,71	59,92	48,34	43,50	42,50	1,40	0,55	16,42	5,84
	10	meadows	1,16	1,19	2,66	2,82	56,39	57,80	45,50	50,50	1,46	0,39	10,89	7,30
	11	maize	1,23	1,27	2,67	2,80	53,93	54,64	39,00	45,50	1,30	0,40	14,93	9,14
Average value			1,15	1,29	2,65	2,78	56,75	53,59	42,66	46,16	1,39	0,45	14,08	7,43

Air capacity in the examined profiles is highly variable. The air capacity in Ah horizon is medium (profiles 3, 10), very high (profiles 2, 8 and 11) and very high (profile 4). In (B) horizon in the profiles 3, 4 and 10 there was a low air capacity, and in profiles 2, 8 and 11 medium air capacity was recorded. The lowest air capacity value in Ah horizon of the sandstones profile was recorded in the profile 3 under maize (10.33 vol. %), and the highest in the profile 2 under meadow (14.00 vol. %). Air capacity is variable which is rapidly changing in the upper layers through tillage, increasing with loosening and reducing compaction due to trampling and natural subsidence. In the profiles of the shales in the Ah horizon the minimum value of air capacity is recorded in the profile 10 under meadow (10.89 vol. %), and the highest in the profile 4 under orchard (16.42 vol. %).

Chemical properties

Parent substrate with its mineral composition is the most important pedogenetic factor which forms chemical properties. Apart from being the result of type of organic matter transformation, the acidic reaction of soil is also the product of parent substrates which are poor with alkali (Knezevic et al., 2011).

On the basis of the recorded values of the pH in 1 M KCl in Ah horizons the analyzed soil was generally very strongly acidic (profiles 4, 8 and 10) and very acidic (profiles 2, 3 and 11). In all (B) horizons of the tested profiles the soil was very acidic (Table 3).

The pH in H_2O and KCl decreases with depth of profiles. The lower average value of pH in H_2O and KCl in Ah horizon was recorded on the shales profiles in relation to the profiles in sandstones. The highest pH value in H_2O and KCl in profiles of the sandstones in the Ah horizon was recorded in the soil under meadow, and then in the soil under the maize, and the lowest pH in H_2O and KCl was observed in the soil under orchard. The highest pH value in H_2O and KCl in profiles of the shales in Ah horizon was recorded in the soil under maize and the lowest in the soil under orchards.

Ah horizon of the analyzed soil is a poor to moderate humus content, while (B) horizon in all tested profile has poor humus content. The average value of humus in the profiles on the sandstone is slightly higher in comparison to the profiles on the shales. The humus content rapidly decreases with depth in all profiles. In the profiles of the sandstones in the Ah horizon the highest humus content was recorded in the soil under orchards (2.35%), and slightly lower in the soil under the maize (2.08%). The lowest humus content is recorded in the soil under meadows (1.74%). In the profiles of shales the highest humus content was recorded in the Ah horizon land under maize (2.33%), as a result of the application of manure and fertilizers. The minimum content of humus in the Ah horizon in the profiles of the shales was recorded in the profile 4 under orchard (1.30%).

Table 3. Chemical properties of soil

Parent subtrates	Profile	Land use	pH in H_2O		pH in KCl		Humus (%)		Total nitrogen (%)		P2O5 mg/100 gr soil		K2O mg/100 gr soil	
			A_h	(B)	A_h	$(B)_h$	A_h	(B)	A_h	(B)	A_h	(B)	A_h	(B)
Sandstones	2	meadows	5,45	4,66	4,64	3,93	1,74	0,30	0,09	0,02	2,30	0,50	18,4	14,3
	3	maize	5,08	4,57	4,56	3,87	2,08	0,32	0,10	0,02	3,26	0,00	27,1	10,5
	8	orchards	4,90	4,67	4,01	3,85	2,35	0,69	0,12	0,03	1,70	0,11	5,30	4,60
Average value			5,14	4,63	4,4	3,88	2,00	0,44	0,10	0,02	2,42	0,20	16,9	9,80
Shale	4	orchards	4,20	4,02	3,54	3,49	1,30	0,15	0,06	0,01	0,62	0,00	9,00	6,60
	10	meadows	5,19	5,31	4,23	4,02	2,02	0,25	0,10	0,01	2,10	0,11	13,4	5,90
	11	maize	5,46	4,83	4,57	3,81	2,33	0,36	0,12	0,02	2,63	0,50	22,9	18,3
Average value			4,95	4,72	4,11	3,77	1,88	0,25	0,09	0,01	1,78	0,20	15,1	10,26

Ah horizon soil profiles are moderately to well supplied with nitrogen, while the (B) horizon is poor with nitrogen. The reduction of total nitrogen with increasing depth is due to a decrease of humus content. Average values of total nitrogen in the studied profiles are uniform in both the horizon profiles on sandstones and shales. In all soil samples, readily available forms of phosphorus are low. The content of available phosphorus in the profiles on sandstones and shales in the Ah horizon is the highest in the profiles under maize, which is the result of fertilization of this soil with phosphorus fertilizers, while the lowest content of available phosphorus is observed in profiles under orchard. Provision of readily available potassium in the Ah horizon is poor (profiles 4, 8), moderate (profiles 2, 10) and good (profiles 3, 11), while in (B) horizon the soil is poorly (profiles 4, 8, 10) to moderately provided (profiles 2, 3 and 11). In average, the content of readily available potassium in Ah horizon is somewhat higher in the profiles on the sandstones compared to the shales profiles, while in (B) horizon the average content of readily available potassium is the higher in the profiles on shales compared to sandstones. The content of easily available potassium decreases with the depth of profile which is, according to Ashok (1998) result of a higher content of organic matter in the Ah horizon in relation to the adjacent horizon and due to the fertilization of the surface horizon. The profiles of the sandstones in the Ah horizon the highest content of readily available potassium was recorded in the profile 3 under maize (27.1 mg/100 g soil), and the lowest in the profile 8 orchard (5.30 mg / 100 g soil). Also, in the Ah

horizon of profile on shales, the highest content of readily available potassium was recorded in the profile 11 under maize (22.9 mg/100 g soil), and the lowest in profile 4 under orchard (9.0 mg/100 g soil). The application of organic and mineral fertilizers, the availability of physiologically active phosphorus and potassium was improved in soil under maize. From the aspect of crop production the soil under maize is well provided with potassium, which means that the application of potassium fertilizers compensated the losses of potassium which resulted from removal of the crop, fixation by clay minerals and leaching. Providing of soil with readily available phosphorus for crop production has remained poor.

Conclusion

Profiles on the sandstones have a lighter texture composition in relation to the profile on the shales as a result of the influence of parent material. The textural composition of the soil affected the water-physical properties of the soil which are favorable for plant production. Chemical properties are characterized by acid reaction of soil. The analyzed soil is poorly to moderately composed of humus in the surface horizon, and in cambic poorly. The content of humus in the Ah horizon profile on sandstones is somewhat higher than in the profile on the shales, while in horizon (B) there is higher humus content in the profiles on shales with respect to the profiles on sandstones. In the profiles on sandstones in the Ah horizon the highest content of humus is recorded in the soil under orchard, followed by soil under maize, whereas in the Ah horizon profiles on the shales, the highest humus content is recorded in the soil under maize. In the surface horizon in the soil under maize in both examined parent materials, the highest content of available phosphorus and potassium is recorded in comparison to the other tested variants of land use. Fertilization increased readily available potassium content in the tested soil, while the availability of phosphorus remains poor.

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THE ANTI-FATTY LIVER EFFECTS OF GUAVA LEAVEAS AND POMEGRANATWE PEEL EXTRACTS ON ETHANOL-EXPOSED RATS

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Abstract

This study is designed to determine total phenols and flavonoids and to evaluate the antioxidant activity of pomegranate peel and guava leaves methanolic extract as well as to assess the effects of pomegranate peel and guava leaves methanolic extracts against ethanol induced fatty liver in wistar rats. Both two extracts showed a good amount of phenols and flavonoids and appeared antioxidant activity in reducing power and total antioxidant capacity assay. Twenty-four male albino rats were used over a 30 day period. The animals were divided into 4 groups, where group (A) represents the negative control which was a fed basal diet, while group (B) administered by stomach tube with ethanol (2g/Kg body weight) to serve positive control. The other two groups were given ethanol supplemented with methanolic extracts of pomegranate peel and guava leaves. Group (C) was treated daily with pomegranate peel extract (200 mg/ Kg body weight), while group (D) was treated with guava leaves (200 mg/ Kg body weight). The plasma lipid profile and liver functions in the different groups were determined. The rats in the treated groups (C and D) showed significantly lower levels of total cholesterol, triglycerides, LDL- cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, ALT, AST and ALP activities as well as high levels of total protein in comparison with the positive control group. Our results suggests that both two plant extracts haves anti fatty liver effect which induced by ethanol treatment, and pomegranate peel extract showed highly significant effects compared with guava leaves extract.

Keywords: *Pomegranate peel, guava leaves, fatty liver, ethanol.*

Introduction

Guava (Psidium guajava) is one of the most effective edible plants and it has been used for a long time in traditional medicine. It has several biological activities such as antidiabetic (Oh et al., 2005), anticough, and antibacterial (Jaiarj et al., 1999). Recently, it has been reported to possess high potential for antioxidant activity (Guo et al., 2003). The leaf is the most effective part of guava plant (Tachakittirungrod et al., 2007).

Pomegranate is an important source of bioactive compounds and it has been used for folk medicine for many centuries. Pomegranate juice has high antioxidant activity and it is effective in the prevention of atherosclerosis (Li et al., 2006). Pomegranate peel extract could effectively protect (after oral administration) against CCl_4 induced hepatotoxicity, in which reactive oxygen species (ROS) damage was intensively involved (Murthy et al., 2002).

Purpose of this research is determination of the antioxidant activity and evaluation of the anti-fatty liver effects of guava leaves and pomegranate peel extracts on ethanol-exposed rats.

Materials and methods

Determination of total phenolic compounds, total flavonoids and reducing power

The amounts of phenolic compounds in different extracts were determined with Folin-Ciocalteu reagent using the method of (Spanos and Wrolstad, 1990) and aluminum chloride colorimetric method was used for flavonoids determination according to (Aiyegoro and Okoh,

2010) while the reducing power of different extracts was determined according to the method of (Ebrahimzadeh et al., 2008).

Biological experiment

Twenty four Male albino wistar rats weighting between 150 - 200 g each were used for this experiment. They were procured from Research Institute of Ophthalmology, Giza, Egypt. The animals were divided into 4 groups with 6 rats in each group. Group (A) served as Control. Group (B) treated with 2g/Kg body weight of ethanol. Group (C) treated with 2g/Kg body weight of ethanol plus 200 mg/Kg body weight of Pomegranate peel extract, and Group (D) received 2g/Kg body weight of ethanol plus 200 mg/Kg body weight of guava leaves extract.
Blood samples were collected after 30 days in tubes contain heparin as an anticoagulant from the eye plexuses under diethyl ether anesthesia and then centrifuged at 3000 rpm for 20 min. to obtain plasma, which was kept frozen until analysis. The total cholesterol (TC) was analyzed calorimetrically according to (Richmond, 1973). HDL - cholesterol was determined according to (Lopez et al., 1977). LDL – cholesterol and VLDL -cholesterol were calculated according to (Friedewald et al., 1972). The triglycerides (TG) were analyzed according to (Bucolo and David, 1973). The both of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activities were measured according to the method described by (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) activity was measured by (Hausamen et al., 1967).Total protein was determined according to (Tietz, 1976), and albumin was determined according to (Doumas et al., 1971).

Statistical analysis

To determine statistical difference between means ($p < 0.01$), ANOVA and Duncan's test were calculated using SPSS statistical software package v.11. Results were expressed as mean values ± SD.

Results and discussion

Total phenolic, total flavonoids contents and in vitro antioxidant activity of plant extracts:

The antioxidant activity of plants is mainly contributed by the active compounds present in them. The phenolic contents of guava leaves and pomegranate peel extracts were found to be 98 and 187 (mg/100g), respectively, while total flavonoids content were 35 and 46 (mg/100g) respectively (Table 1). These results of pomegranate peel is agree with (Li et al., 2006) who determined total phenols and total flavonoids of Pomegranate peel and pulp, while guava leaves results is agree with (Nantitanon et al., 2010) who studied total phenols in different guava leaves extracts.
From our data we can reported that methanolic extract of Pomegranate peel have high concentration of both total phenolic compounds and total flavonoids compared with the methanolic extract of guava leaves.

Table 1. Total phenolic and total flavonoids contents of guava leaves and pomegranate peel extracts

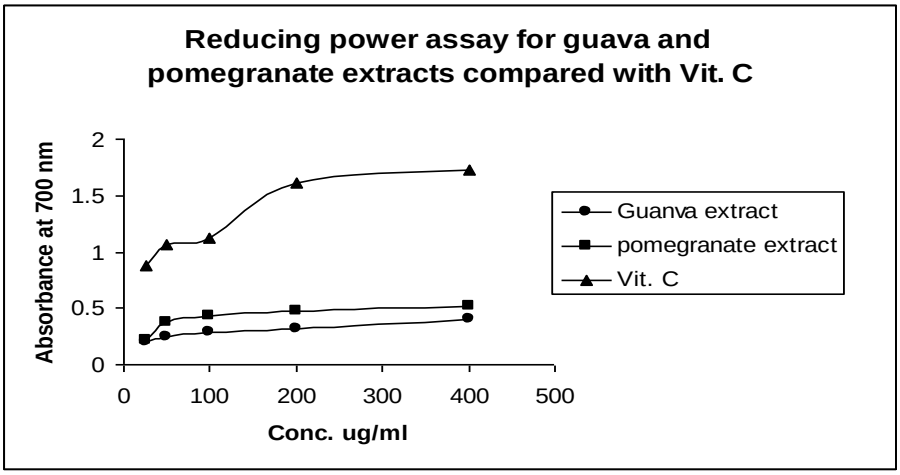
Plant extract	Total phenolic content (mg/100g)	Total flavonoids content (mg/100g)
Methanol extract of guava leaves	98	35
Methanol extract of Pomegranate peel	187	46

Figure (1) showed the dose– response curves for the reducing powers of the guava leaves extract and pomegranate peel extract. It was found that the reducing powers of extracts also

increased with an increase in their concentrations. At the highest concentration (400 ug/ml) pomegranate peel extract showed highest activity (0.521) while guava leaves extract was (0.4), at the same concentration.

Pomegranate peel extract evaluated by Ferric reducing antioxidant power (FRAP) assay, was found to be the richest source of antioxidants among peel extracts of the most commonly consumed fruits. Similarly, pomegranate peel extract demonstrated 2.8-fold higher antioxidant activity compared to pomegranate seed and leaf extracts (Tehranifar et al., 2011).

Fig. 1: Reducing power assay for guava leaves and pomegranate peel extracts



Effect of pomegranate peel and guava leaves extracts on liver function in ethanol-treated rats

The results of biochemical indicators of liver function are summarized in Table (3). The administration of ethanol caused severe hepatotoxicity in the rat, as evidenced by the significant elevations of plasma total bilirubin, direct bilirubin content, aminotransferases (ALT and AST) and ALP activities in experimental rats compared with normal control group. The levels of plasma total bilirubin and direct bilirubin in the plasma of the ethanol treated group were 1.6 and 0.8 mg/dl, respectively. Also the activities of ALT, AST and ALP in the same group were 96.5, 88.43 and 79.34 U/L, respectively. However, the activities of ALT, AST, and ALP in rats administrated with pomegranate peel and guava leaves extracts were significantly decreased. Also our data sowed significant decreased in both plasma total protein and albumin levels in ethanol treated group compared with control. The levels of total protein and albumin in the plasma of the ethanol treated group were 4.07 and 2.28 g/dl respectively. However, the levels of total protein and albumin in rats administrated with pomegranate peel and guava leaves extracts were significantly increased.

Ethanol-induced liver damage progresses through alcoholic fatty liver, alcoholic hepatitis, fibrosis and cirrhosis (You et al., 2010). Liver damage due to ingestion of alcohol is a well known phenomenon in animals. It is well-established that ethanol inhibits the mitochondrial electron transport chain, resulting in increased ROS production (Song et al., 2008). So the oxidative stress plays an important role in the development of alcohol liver disease (Yurt and Celik, 2011). Hepatic injury also results in the leakage of cellular enzymes into plasma (Baldi et al., 1993).

Increased activities of serum enzymes such as ALT and AST and decreased levels of albumin have been observed in alcohol-treated mice or rats, indicating increased permeability, damage, steatosis and inflammation (Ki et al., 2007).

Our results showed that pomegranate peel and guava leaves extracts treatments protect the liver by significantly inhibit the ethanol-induced ALT, AST and ALP activities. At the same

time increases total protein and albumin levels. And we can notice that pomegranate peel extract showed highly significant effect compared with guava leaves extract due to highly phenolic and flavonoids contents and highly antioxidant activities

Table 3. Effect of pomegranate peel and guava leaves extracts on liver function in rats treated with ethanol for 30 days.

Liver function	Group A	Group B	Group C	Group D
ALT (U/L)	34.24 ± 2.96 a	96.5 ± 3.1 d	64.83 ± 3.6 b	75.47 ± 1.28 c
AST (U/L)	22.87 ± 2.08 a	88.43 ± 1.4 d	45 ± 2.58 b	54.75 ± 2.68 c
ALP (U/L)	33.41 ± 3.6 a	79.34 ± 5 d	52.61 ± 2.16 b	62.63 ± 2.15 c
Total protein (g/dl)	5.69 ± 0.17 d	4.07 ± 0.1 a	4.94 ± .065 c	4.55 ± 0.96 b
Albumin (g/dl)	3.64 ± 0.22 c	2.28 ± 0.046 a	3 ± 0.061 b	3.2 ± 0.02 b

Values represents mean ± standard deviation (6 rats).

(a, b, c, d) means in the same column followed by the same letters do not differ significantly,

and when the values followed by different letters differ significantly at $p \leq 0.01$.

Effect of pomegranate peel and guava leaves extracts on plasma lipid profiles in ethanol-treated rats:

Accumulation of fat is the earliest and most common response to heavy alcohol intake. Alcoholic fatty liver is usually characterized by the enlargement of the liver, the increase of the serum and hepatic TG levels, together with a lot of fat droplets in the liver sections.

Plasma triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol levels and risk ratio were significantly increased in ethanol administered rats compared to that of control animals. In rats co-treated with pomegranate peel and guava leaves extracts along with ethanol all these parameters levels were significantly reduced compared to rats treated with ethanol alone (Table 4). Although our data appeared that, pomegranate peel extract showed highly significant effect compared with guava leaves extract and we can notice that clearly in risk ratio which showed not significant changes between co-treated pomegranate peel group and normal control rats.

In recent years, the positive effects of flavonoids on human health have attracted more attention. Especially, quercetin, a flavonoid found in many plants, is widely distributed in fruits and vegetables. Most of the biological actions of quercetin seem to be associated with its potency as an antioxidant. The antioxidant effect of quercetin has been reported by different studies (Kalender et al., 2012 and Sanchez-Gallego et al., 2011).

This is in accordance with the previous study where flavonoids presented the largest percentual reduction of cholesterol in the triton induced hyperlipidemic rats (Ricardo et al., 2001), and restores the lipid profiles to normal (Krishnaveni et al., 2010). The hypolipidemic effect may be due either to the retarding effect on lipid fraction absorption or the increase in the LDL receptor mediated cholesterol rein oval (Lieber, 1994).

Table 4. Effect of pomegranate peel and guava leaves extracts on plasma lipid profiles in rats treated with ethanol for 30 days

Lipid profiles	Group A	Group B	Group C	Group D
Triglycerides (mg/dl)	119.3 ± 6.16 a	213.05 ± 2.9 d	168.6 ± 5.79 b	192.8 ± 3.69 c
Total cholesterol (mg/dl)	89.5 ± 1.72 a	194.5 ± 5.86 d	123.25 ± 6 b	147.7 ± 5.55 c
HDL-cholesterol (mg/dl)	32.4 ± 0.57 a	52.76 ± 2.8 c	42.6 ± 1.8 b	46.28 ± 0.56 b
LDL-cholesterol (mg/dl)	33.28 ± 2.6 a	99.17 ± 4.5 d	46.9 ± 4.7 b	62.8 ± 6.4 c
VLDL-cholesterol (mg/dl)	23.86 ± 1.23 a	42.6 ± 0.59 d	33.73 ± 1.16 b	38.57 ± 0.74 c

Values represents mean ± standard deviation (6 rats).
(a, b, c, d) means in the same column followed by the same letters do not differ significantly, and when the values followed by different letters differ significantly at $p \leq 0.01$.

Effect of pomegranate peel and guava leaves extracts on liver triglycerides and total cholesterol in ethanol-treated rats:

The tissue total cholesterol and triglycerides levels were found to be significantly increased in rats administered ethanol alone. Rats co-treated with pomegranate peel and guava leaves extracts along with ethanol registered a significant decreased in tissue cholesterol and triglycerides levels compared to rats treated with ethanol alone (Figure 2). The hypolipidemic effect may be due prevention of liver from damage caused by ethanol dose.

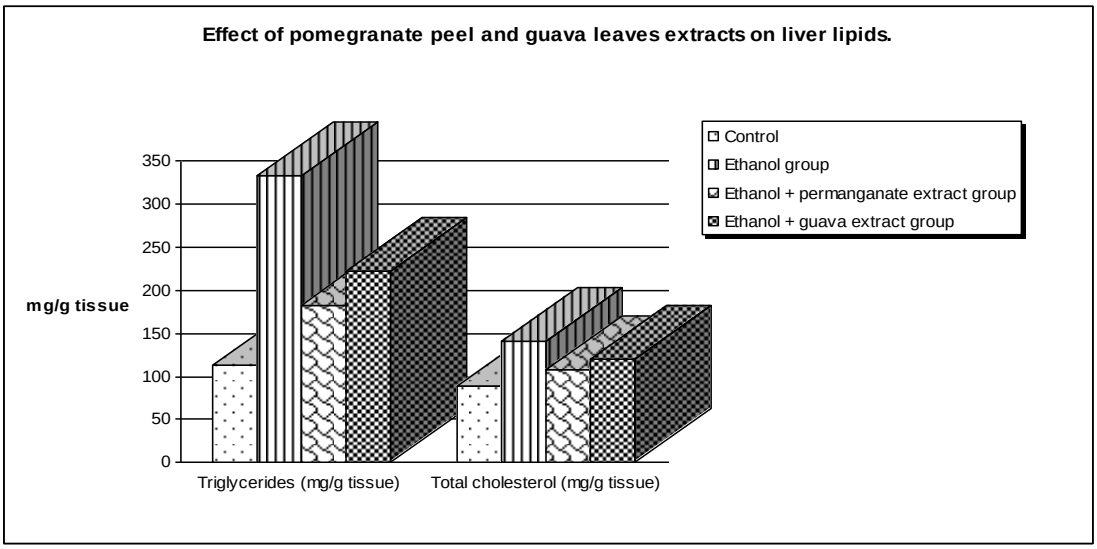


Fig.2. Effect of pomegranate peel and guava leaves extracts on liver lipids in rats treated with ethanol for 30 days.

Conclusion

From our study it is obvious that ethanol induced alteration in the lipid profile, and liver damage can be ameliorated by pomegranate peel and guava leaves extracts co-treatment and

pomegranate peel extract showed best effect. Our study advocates the use of natural plant extracts (pomegranate peel and guava leaves) in diet to reduce toxic effects of ethanol.

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Original scientific paper
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VERTICAL MIGRATION OF ^{90}Sr RADIONUCLIDE IN AGROECOSYSTEM

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Abstract

The present study was conducted in order to determine ^{90}Sr radionuclide migration in the agricultural soil within the long-term field experiment at the Rimski Šančevi in Novi Sad (Republic of Serbia). This radionuclide is one of the most hazardous fission products. The downward migration of ^{90}Sr anthropogenic radionuclide in soil is a key factor in the understanding of behavior of this radionuclide in natural systems. The validated radiochemical analytical procedure for separation and determination the activity of ^{90}Sr in soil samples was applied. The measurements were performed by α/β low level proportional counter. The activity concentrations of ^{90}Sr at 4 sampling locations and 3 depths of soil are reported. The obtained results showed that the activity concentration of ^{90}Sr in the chernozem soil ranged from 0.2 (minimum detectable activity) to 1.1 Bq kg^{-1}. It was concluded that exists uniform distribution of ^{90}Sr in agroecosystem of the chernozem zone. Also, the obtained results showed that the highest concentrations of ^{90}Sr radionuclide were found in the arable horizon.

Keywords: *^{90}Sr radionuclide; soil; agroecosystem*

Introduction

^{90}Sr is the artificial long-lived radionuclide, so-called fission product created in nuclear reaction (^{235}U (n,f) ^{90}Sr), which is released in the environment not only from global fallout, already and accidents involving nuclear materials (Kavasi et al., 2015). This radionuclide belongs to the group of the extremely toxic radionuclides and it is one of the most significant radioecological factors because it has a high fission yield, a long physical (28.6 y) and biological half-life (Grahek and Rožmarić-Mačefat, 2005; Friberg, 1997). Thus, the investigation of ^{90}Sr translocation in the environment is very important for determination of radiobiological effects in each part of ecosystem. The artificial radionuclide once released in the environment can find their way into the food-chain leading to contamination of food (Sandeep et al., 2009).

The downward migration of anthropogenic radionuclides in the soil depends on several factors such as the way and chemical form in which they were deposited on the soil, the climatic conditions of the ecosystem, soil characteristics (Guillen et al., 2015). One of the major factor of influence on radionuclide mobility in soil is the pH value of soil (Koch-Steindl and Prohl, 2001). Sorption on clay minerals, oxides and organic matter are the main processes that have an impact on radionuclide mobility in soil (Vukašinović et al., 2010).

According to the degree of mobility in the soil, anthropogenic radionuclides with long half-life can be divided into the following series: ^{90}Sr>^{106}Ru>^{137}Cs>^{144}Ce>^{129}I>^{239}Pu. For contamination of soil and live organisms, hazard effects have only radionuclides with a long biological half-life, of which the most significant are ^{90}Sr and ^{137}Cs. Sorption of these

radionuclides in the soil has a great significance, because of sorption determines their migratory ability, as well as their penetrating in the root system of plants (Gajić, 2006).
The main objective of this study is to analyze the downward migration of ^{90}Sr in soil of experimental field in the northern province of the Republic of Serbia. A tendency for the accumulation of ^{90}Sr was observed in the soils subjected to a long-term fertilization with NPK. This study will contribute to the better understanding of ^{90}Sr migration in the agricultural managed systems.

Material and Method

In order to measure the content of ^{90}Sr, the soil samples were collected from a long-term experiment (LTE) „Plodoredi“ at the Rimski Šančevi experimental field of the Institute of Field and Vegetable Crops in Novi Sad (45^{o}19’ N, 19^{o}50’ E, *Figure 1*).

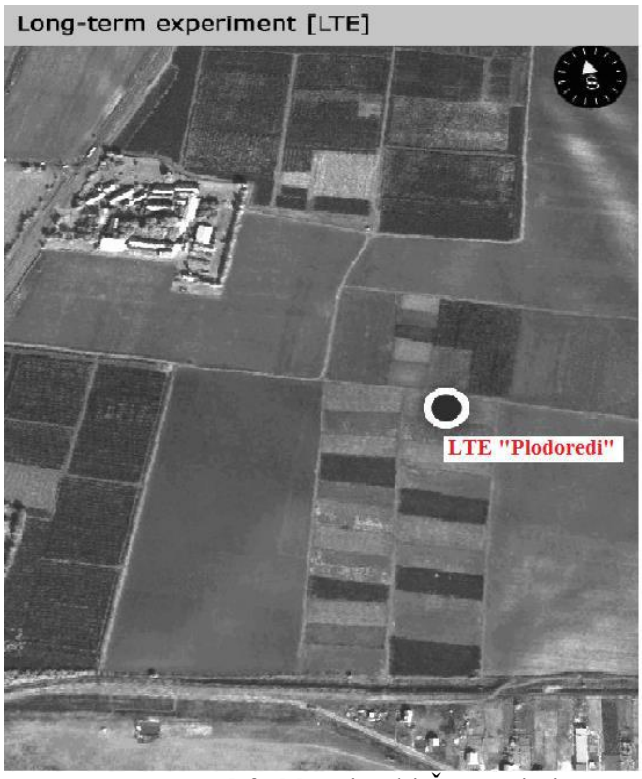

Figure 1. Experimental field „Rimski Šančevi“ in Novi Sad

The soil sampling was conducted in the autumn season of 2013 year. The each sampling locations and ambient dose rates are given in *Table 1*. The measurement of the ambient dose rate at sampling locations was performed using a scintillation probe (Model 6150 AD-b produced by Automess, Germany) 1 m above the ground.

Table 1. The sampling locations and the corresponding ambient dose rates

Location code	Treatment of soil	Ambient dose rate (nSv h^{-1})
L1	2-year crop rotation (maize-**winter wheat**) from 1970 120 kg ha^{-1} N per year+PK	125
L2	2-year crop rotation (**maize**-winter wheat) from 1970 100 kg ha^{-1} N per year+PK	105
L3	**Winter wheat** monoculture from 1970 100 kg ha^{-1} N per year+PK	110
L4	2-year unfertilized crop rotation (winter wheat-**maize**) from 1946	100

About 2 kg of soil samples were taken from three depths (0-10, 10-20 and 20-30 cm) at each sampling location. This soil is slightly alkaline with nearly equivalent percents of fine sand and silt.

The preparation of soil samples involved the following: removing plant residues from the basic soil mass, samples drying at 105 ^{0}C during 24 h and sieving through a stainless steel sieve (diameter 3 mm) followed by grinding to powder (according to MARLAP 2004). 300 g of dry soil was mineralized at 500 ^{0}C during 24 h by the method of dry burning for further radiochemical analysis of ^{90}Sr.

The procedure of validated radiochemical analytical method (Sarap et al., 2014) was applied for the determination of ^{90}Sr activity concentration in agricultural soil samples. This method is based on the radiochemical separation of ^{90}Y from the sample using aluminium collector. After the establishment of radioactive equilibrium between ^{90}Sr and daughter radionuclide ^{90}Y during 18 days, ^{90}Sr is determined via ^{90}Y. The measurements were performed using α/β low-level proportional counter Thermo Eberline FHT 770 T (ESM Eberline Instruments GmbH, Erlangen, Germany).

The activity concentration of ^{90}Sr (*A)* expressed as Bq kg^{-1}, was calculated using the Equation:

$$A = \frac{(N-B)\times \exp\left(\frac{\ln 2 \times t}{T_{½}}\right)}{P_{Al}\times P \times Ef \times m} \qquad (1)$$

where N is the count rate of the sample (s^{-1}), B denotes the background (s^{-1}), t - the time elapsed from ^{90}Y separation (h), $T_{1/2}$ is the half-life of ^{90}Y (h), P_{Al} - the yield of aluminium which is determined for each sample separately, P - the yield of the used method, Ef - the efficiency of the detector and m is the mass of the sample (kg).

Results and discussion

Agricultural soil which is the subject of this investigation belongs to the Haplic Chernozem (CHha) type (typization according to IUSS Working Group WRB, 2006) and its chemical reaction is slightly alkaline. In 4 experimental variants with different levels of fertilization (120 kg ha^{-1} N per year+PK, 100 kg ha^{-1} N per year+PK and without of fertilization, marked as L1, L2, L3 and L4 in *Table 2*), 2-year crop rotation (maize-winter wheat) and winter wheat monoculture were included. The ^{90}Sr activity concentrations for different depths of investigated soil are presented in *Table 2.*

Table 2. The ^{90}Sr activity concentration (*A*) in agricultural soil, expressed on dry matter.

Location code	Depth of soil (cm)	A (Bq kg^{-1})
L1	0-10	1.1 ± 0.4
	10-20	0.6 ± 0.1
	20-30	< 0.8
L2	0-10	0.8 ± 0.2
	10-20	0.6 ± 0.1
	20-30	< 0.2
L3	0-10	0.4 ± 0.1
	10-20	0.5 ± 0.1
	20-30	0.4 ± 0.1
L4	0-10	0.5 ± 0.1
	10-20	0.5 ± 0.1
	20-30	0.4 ± 0.1

The obtained values of ^{90}Sr activity concentration in agricultural soil are ranged from 0.4 to 1.1 Bq kg^{-1} and 0.5 to 0.6 Bq kg^{-1} for 0-10 and 10-20 cm depths of soil, respectively. The ^{90}Sr activity concentrations for 20-30 cm depth of soil at locations 1 and 2 are below of the minimal detectable activity, while for locations 3 and 4, the values were identical and amounted to 0.4 Bq kg^{-1}. The differences in activity concentrations of ^{90}Sr radionuclide by the soil layers are evident for locations 1 and 2, while for locations 3 and 4 are insignificant. Anyhow, the highest content of ^{90}Sr in investigated soil profiles is obtained for location 1, where the maximum quantity of fertilizer is used. Based on the obtained results it can be seen that the distribution of ^{90}Sr is uniform in examined agricultural soil profiles. Also, it can be said that ^{90}Sr shows tendency to retention in the upper soil layers, apropos in arable horizon.

Conclusion

The downward migration of anthropogenic radionuclide of ^{90}Sr in agroecosystem was analyzed. Investigation of the vertical migration of ^{90}Sr radionuclide in agricultural soil showed uniform distribution. The highest ^{90}Sr activity concentrations are obtained in topsoil layer of the chernozem at 0-10 cm of depth. The obtained results are confirmed the literature data that the most of ^{90}Sr retains in the arable horizon of agricultural soil.

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Review paper
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ADDRESSING WATER SCARCITY THROUGH RECYCLING AND MAKING THE BEST USE OF WASTE WATER

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Abstract

As world demand for water grows, water reclamation and reuse becomes increasingly important and an indispensable component of integral water resources management to enhance water supply reliability. Now a days, politically and technically it is well recognized the important role the treated waste water and its reuse could play in reducing the enormous gap between the increasingly water demand and the limited fragile water supply particularly in many arid and semi arid developing countries. In the future, it is certain that water reuse will increase worldwide not only with a view to countering problems already known, but also to counter a new threat arising from climate change and the expected changes it could have on the available water resources. (IPCC 2007). Water reuse is meant to help close the water cycle and therefore enable sustainable reuse of available water resources. When integrated to water resources management, water reuse may be considered as an important component of the national research policy and as an integral part of environmental pollution control and water management strategy. It may present benefits to public health, the environment and economic development. Reclaimed water may provide significant additional renewable, reliable amounts of water and contribute to the conservation of fresh water resources. To obtain maximum benefits and to minimize the risks from water reuse, we are in need to better understanding of the advantages, the drawbacks and controversial opinions about planning, methodologies, appropriate technologies, implementation challenges and opportunities. Those are some of the fundamental issues beside others to be fully discussed in this paper.

Keywords: *waste water, water scarcity, water reuse, water management and research policy*.

Introduction

Nowadays, politically and technically the important role of treated wastewater and its reuse could play in reducing the enormous gap between the increasing water demand limited fragile water supply particularly in many arid and semi arid developing countries is well recognized. In dead for some water scarce regions including those of the Mediterranean and the Middle East, treated wastewater is becoming a common source for additional water and is already included in their master plans (Bahri, 2002; Bazza, 2002 and Hamdy, 1999).As a matter of fact, for many countries, wastewater reuse is recognized as an encouraging solution to cope with the problem of water scarcity, the big challenge many countries are now facing (Collinos *et al.,* 2009). In order to cope with water scarcity, one of the most promising supply management practices to be explored is the reuse of wastewater (Periera et *al.,* 2002). Technological advances in the treatment field have enabled treatment facilities to obtained reclaimed water quality suitable for urban and even potable supply (Maliva and Missimer, 2012). However, several obstacles have hampered the implementation of this type of projects for instance, the tight compartmentalization of sanitation and supply sectors has limited the development of reuse schemes and consequently resulted in a mismanagement of water resources. Bridging supply and sanitation sectors into a more integrated approach is vital for achieving sustainable management of urban water systems (Lazarova *et al.,* 2001; Bixio *et al.,* 2006). With issues of climate change, increase in urban population and increased demand for

water from competing sectors, wastewater recycling is becoming an important strategy to complement the existing water resources for both developing and developed countries and there are lessons, experiences data and technology that can be shared for mutual benefit.

Wastewater: a growing source

By 2015, 88% of the one billion person growth in the global population will occur in cities, the most majority of this growth will occur in developing countries (UNDP, 1998). An increase in urban water supply ensures an increased wastewater generation. The growing wastewater volumes render a cheap and reliable alternative to conventional irrigation systems. In this context wastewater is a resource that could be of increased national and global importance, particularly in urban and peri-urban agriculture. According to Hussein *et al*., (2001), there is at least 20 million hectares in 50 countries irrigated with raw or partial treated wastewater. After reviewing many overseas recycling projects, Radcliffe 2004 concluded that worldwide, water reuse is becoming an increasingly common component of water resources planning as the costs of wastewater disposal rise and the opportunities for conventional water supply development dwindle. In many developing countries particularly those in arid and semi arid regions the use and recycling of wastewater is always increasing with time due to a combination of factors among them: urban population increase, decrease in rainfall, water scarcity, environmental concerns, need for greener water strategies and improved technology. **Figure (1)** illustrate the increase in urban water supply coverage have been and will continue to be the highest in Asia and Africa, where absolute population figures as well as population growth are the highest. In this context wastewater is a resource that could be of increased national and global importance.

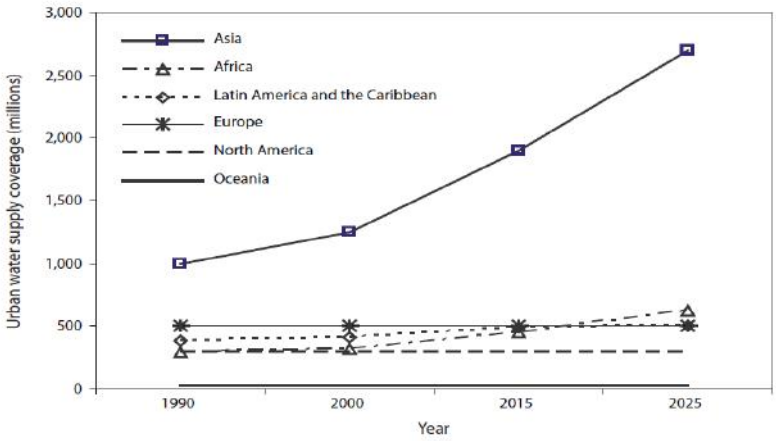

Figure 1. Growth in urban water supply coverage by regions of the world.
Source: Scott et al., 2004

Water reuse: the main drivers

Main drivers for reusing water are: drought and scarcity of water supplies; resource efficiency and protection of existing water supplies.

Drought and water scarcity: treated wastewater is commonly used for irrigation purposes around the world due to drought and scarcity of water supplies. This is the case in Europe and especially in the southern Europe as well as the arid and semi arid countries of the Mediterranean and Middle East regions.

Resources efficiency: Not all water that is consumed in industry, agriculture or in municipality needs to be drinking water quality. Producing clean water from waste water in the long term can be more economical and sustainable than over consuming fresh water sources or desalinating sea water. Treated wastewater can be used in many applications. For instance in the pulp and paper industry the water is reused several times and water

consumption has dropped significantly over the years. In the 1970s producing 1 tonne of pulp required as much as 250 m^3 of water – now it only takes 5-50 m^3. The past decade saw water consumption of paper machines being reduced by 1/3 as a result of more efficient water circulation.

It is well recognized that, insufficient financial and coping capacities constrain the establishment of comprehensive wastewater management systems The use of untreated wastewater is not limited to the countries and cities with the lowest gross domestic product (GDP), but is also a common practice in many middle-income countries as well (Raschid-Sally and Jayakody, 2008).

Wastewater reuse: the benefits

The most relevant reuse benefit of wastewater is making a new water supply source available. Besides, this new resource guarantees a high level of supply relatively constant between years (Friedler, 2001), which may bring increased benefits to users that suffer from constant water shortages (Mesa- Jurado *et al.*, 2012). Increasing the resource availability entails decreasing on water stressed bodies (Miller, 2009). The reuse of treated wastewater can provide the following benefits:

Economic benefits: Designed correctly, recycling can help achieve considerable savings because water only needs to be treated for its purpose. For example, desalinating sea water for irrigation would be more energy demanding and expensive than using reclaimed water for the same purpose. **Social benefits**: Water reuse can ensure the safeguarding of existing water supplies and to manage the imbalance between water demand and supply. **Ecological benefits**: Water reuse can help to decrease the amount of waste water discharged into the environment. Ground water levels can be kept; overutilization of water from lakes can be avoided and in some cases nutrients in wastewater can be directly reused in agriculture. Direct wastewater reuse provides also an effective means of coping with nitrogen and other nutrients and pollutants present in effluents (Hernandez-Sancho *et al.*, 2010). At the same time, direct wastewater reuse may reduce the dependency on other sources of fertilization (Toze, 2006, and Fonder *et al.*, 2010). **Environmental benefits:**In addition to providing dependable, locally controlled water supply, water recycling provides tremendous environmental benefits including the following:

- Decrease diversion of freshwater from sensitive ecosystems: people who reuse water can supplement their demands by using a reliable source of recycled water, which can free considerable amounts of water for the environment and increase flows to vital ecosystems and thereby avoid the diversion of freshwater from sensitive ecosystems that can cause deterioration of water quality and ecosystem health.
- Enhance wetlands and riparian (stream) habitats: experiences gained demonstrate clearly that streams that have been impaired or dried from water diversion, water flow can be augmented with recycled water to sustain and improve the aquatic and wildlife habitat.
- Prevent and reduce pollution: A clear demonstration for such benefit can be seen with recycled water containing higher levels of nutrients, such as nitrogen. Application of recycled water for agricultural and landscape irrigation can provide an additional source of nutrients and lessen the need to apply synthetic fertilizers (El-Motaiam and Abdel Monem, 2001).
- Saving energy: recycling water on site or nearby reduces the energy needed to move water longer distances or pump water from deep within an aquifer. Tailoring water quality to a specific water use also reduces the energy needed to treat water using recycled water that is of lower quality for uses that don’t require high quality water saves energy and money by reducing treatment requirements.

In addition, to the environmental benefits achieved due to the reuse and recycling of wastewater, other several benefits could be obtained including:

1. Conservation of fresh water resources for other uses.
2. Reduction in the need for wastewater infrastructure for treatment and disposal of sewage
3.Reduction in the need for nitrate and phosphate removal by the treatment system since these elements have added value in agriculture (Hamdy and Ragab, 2005); Papadopoulos (1995).
4. Reduction of pollution from disposal of sewage into the environment (Fridler, 2001).
5. Combating desertification (Al-Shreideh, 2001).
However, in spite of the benefits gained by reusing treated wastewater in irrigation, there are associated health risks due to contact with reclaimed wastewater. Consequently, it is of utmost importance to make sure that these risks are minimized and monitored (Toze, 2006).

Wastewater use: The risks.

Hussain *et al.,* (2001) developed an overview of the potential benefits and risks arising from the use of wastewater in agriculture. Selected potential impacts are summarized as follows: Public health: Wastewater has the potential to cause diseases as it contains bacteria, viruses and parasites. Also, the inclusion of heavy metals in wastewater can be very dangerous for human health. Wastewater use in agriculture causes risk to the population living within and outside the wastewater irrigation zone. **Crops:** Wastewater is attractive and economically valuable for farmers because it contains important nutrients for crop growth. However, a high concentration of chemical pollutants in wastewater may be toxic to plants. **Soil resources:** Accumulation of nitrogen, phosphorus, dissolved solids and other constituents such as heavy metals in the soil affect its productivity and the sustainability of land use for agriculture. Salt accumulation in the root zone may have harmful impacts on crop yields. **Groundwater resources:** Leaching of nutrients and salts included in wastewater has the potential to affect the quality of groundwater. The degree of impact depends on several factors, including the quality of groundwater, depth of water table, soil drainage and the amount of wastewater applied for irrigation. **Ecological impacts:** Drainage of wastewater from irrigation schemes into water bodies may indirectly affect aquatic life and negatively influence overall biodiversity, e.g., the presence of water birds. **Social impacts:** The use of wastewater in agriculture has different social impacts such as food safety, health and welfare, impaired quality of life, loss of property values and sustainability of land use.

Wastewater – reuse and recycling: Environmental and public health

The main issue derived from wastewater reuse is the health and environmental risk in agriculture and urban uses or ecosystems enhancement purpose. Reclaimed wastewater involves a certain amount of risk because it may contain dissolved solids, heavy metals, pesticides, pathogens and other substances which may cause damage to ecosystems, crops or human beings (Norton-Branduo *et al.,* 2013). In regard to urban uses pathogens (bacteria, virus, protozoa, and parasitic worms) are the most concerning risk since this can cause disease spread. It is worthy to emphasize that an important parameter to decide on the economic feasibility of a reuse plan in agriculture is salinity, as this is not reduced by regular treatments, but requires environmental and economic costly solution such as reserve osmosis (Haruvy, 1998). The impact of effluent disposal on ecosystems has been well documented (Garcia and Pargament 2015). The presence of organic and inorganic nutrients can cause eutrophication and algae bloom, a worldwide environmental problem that causes hypoxia and fish kills, as well as a nuisance to city dwellers and visitors (Gibbs *et al.,* 2002). Beside nutrients, emerging contaminants such as organic wastewater derived compounds might be recognised as one of the most relevant stressors in ecological rehabilitation (Plumlee *et al.,* 2012).

Wastewater recycling: the barriers

A number of factors influence wastewater recycling. These include:

Centralized wastewater treatment systems, the location of the treatment plants, the availability of space in and around cities and the topography – all of these factors restrict the use of wastewater to certain areas and for specific purposes..

There are substantial barriers to entry in the field of wastewater recycling. Wastewater is often operated and owned by a single entity, like the Water Board or sewage treatment plant, which is often the retailer. Also, wastewater recycling often requires a dual reticulation system that is inefficient to duplicate (Muir, 2006).

There are both positive and negative externalities associated with wastewater recycling. The positive externality is: environmental benefits from reduced discharge of saline wastewater into natural water bodies. The negative externalities include potential groundwater pollution and increase in soil salinity if used for irrigation and potential unknown ill effects on human health if used for potable uses..

Public perception is the main barrier for a reuse scheme implementation (Miller, 2006). Without the acceptance of the public, it would be complicated for any utility to locate, finance develop and operate any reclamation plant for the purpose of water reuse (Bdour *et al.,* 2009)

The wastewater challenges

In many parts of the world, wastewater is already used for agriculture. This practice should be encouraged, but, it must be done safely. The challenge is to establish appropriately national and regional guidelines for wastewater reuse in view of the characteristics of the wastewater and the prevailing socio, economic and environment prevailing conditions. Furthermore it is needed to identify practical and safe uses that do not threaten those communities which are dependent on wastewater, accounting the importance that this resource plays in achieving food security in growing urban areas. The fact that right now more than 90% of world's wastewater in discharged untreated into oceans, rivers, or wherever else it can go is indeed a disaster in slow motion that will grow in proportion and impact. In this context the challenge is having solutions for wastewater management not only of human sewage, but also of industrial, agricultural and urban wastewater. According to the World Bank, "The greatest challenge in the water and sanitation sector over the next two decades will be the implementation of low cost sewage treatment that will at the same time permit selective reuse of treated effluents for agricultural and industrial purposes" (Green Arth, 2012).

Wastewater reuse and recycling: supportive policy and institutional setting.

The divers impacts that wastewater use has on the environment, public health, local economies and food security, combined with substantial secondary impacts, highlight the complexity and cross-cutting nature of wastewater management. Proper wastewater management requires collaboration and dialogue between partners and stakeholders involved in wastewater issues, for example, farmers, public health officials, municipal and waste managers, planners and developers. The management of wastewater use in agriculture involves many of the following actors which need to cooperate these actions and regulations: ministers of water resources, agriculture, health, the environment, energy and development; research institutions and universities, NGOs, farmers' groups, consumers, water operators and municipal and local water management institutions. To facilitate the safe management of wastewater in agriculture in this complex context, appropriate policies, legislation, institutional frameworks and regulations at international, national and local level need to be in place which brings these actors together. Scountries have already established platform for these actors to exchange knowledge at national level, there is little structure and opportunity for across sectoral approach and issues of safe use wastewater for agriculture. Important aspects to address this gap such be analysed such as the followings: institutional roles and responsibilities, economic instruments, and education and social awareness.

Concluding remarks and recommendations

Technology selection should focus on the type of reuse anticipated, proximity to source of effluent, environmental sustainability, local conditions, and affordability. Simple solutions that are easily replicated, that allow further up-grading with subsequent development and that can be operated and maintained by the local community are often considered the most appropriate and cost effective

An important element in sustainable water reuse is the formulation of a framework of realistic, achievable and enforceable standards for treated wastewater quality and applications. Monitoring and evaluation of water reuse programs and projects are fundamental and thus must overcome challenges of weak institutions, shortage of trained personnel, lack of monitoring equipment and the relatively high cost required for monitoring processes.

The establishment of clear policies with regard to wastewater management and water reuse is required in order to guide programs, projects and investments relating to wastewater collection, treatment, reuse and disposal in a sustainable manner. These policies should be compatible with other local policies including those on national water management and irrigation, health, sanitation, agriculture and the environment. Policies of water reuse and strategies for its implementation should be part of water resources planning at the national level. At the local level, individual reuse projects should be part of the overall river basin planning effort.

The responsibilities of the various organizations involved in wastewater collection, treatment and reuse must be considered and reconciled by improving coordination among stakeholders and the donor community. Improved participatory approaches (especially with farmers) are further needed including raising the awareness of the general public on the benefits of water reuse.

Furthermore efforts are needed to allocate the required funds to support applied research to find sustainable wastewater treatment processes adaptable to the socioeconomic and climatic conditions.

That the safe use of wastewater in agriculture is an important water resource issue that needs to be addressed, efforts are still needed to advance it in national policies and to implement safe use guidelines and practices. The lack of implementation of guidelines and safety standards can lead to another wise avoidable aggravation of health risks that could result in significant secondary impacts.

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INFORMATION AND COMMUNICATION TECHNOLOGIES IN SMART WATER MANAGEMENT

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Abstract

For most Countries in arid and semi arid regions including those of the Mediterranean water scarcity and water stress are among the dominant crucial problems, seriously affecting any type of development. Indeed, the majority of those countries are subjected to both economic and physical water security. Institutional constraints, aging infrastructure, ICTs gap, lack of investment, poor data and lack of quality services are just some key challenges that the water sector faces. Smart water management (SWM) has become a key policy issue for the 21st century, as a solution to a number of a factors those are impacting the delivery of water to the affected people. Moreover a number of issue linked to climate change, such as lengthy drought and extreme weather variations are worsening the situation. SWM seeks to alleviate challenges in the water sector by promoting the coordinated development and management of ICT products, solutions and systems in order to maximize economic and social welfare without compromising the sustainability water as a resource of the environment. Today ICTs have become pivotal in very day life. Though there is many initiatives designed to enhance water sustainability, efficiency and accessibility via ICTs to address the water crisis and the increasingly surrounding problems. Nowadays ICTs, therefore, are increasingly becoming a key enabling tool for data acquisition, early evaluation, communication or automated management in the water sector. However, in spite of the progress in promoting the integration of ICTs in water resources management yet, there is still a need for the development of standards policies and proper ICTs governance to ensure that there is integrity in the water resources management.

Key words: *Smart water management, ICTs, Security, Sustainable development, Mediterranean*

Introduction

Today, we are faced with an unprecedented number of global water issues. As a solution to these, SWM in connection with ICTs has emerged as stable and efficient water management system. However, for the realization of SWM, it is needed to move forward and strengthen and expand the cooperation systems the implementation of water technology standardization in order to respond effectively and accelerate the resolution of global water issues (ITUT, 2010), Vital solution to relieve water stress caused by climate change and pollution growth can be achieved by applying state-of-the-art technologies using ICTs. SWM could have an important role to resolve water issues by intelligent integrated-technologies and water information generated in the process of the whole water cycle (Al Sheikh, 2011). In addition, it can maximise the efficiency of water management in terms of water resource management, treatment, distribution and energy consumption. The world at it was known, 30 years ago is not what it is today; neither will be the same 30 years from now (FAO, 2011; WWAP 2009; UNEP, 2008). Such dramatic change in such a short period of time is mainly attributed to the develop0ment of ICTs. Modern ICTs have provided today's society with a vast array of innovative communication capabilities and have transformed the world into a global village.. Harnessing ICTs technology within the water sector creates a more intelligent means to manage and protect the plant's water resources (DeLange *et al.*, 2013). Indeed ICTs can play

a special role in this respect through a number of technologies that help in better distribution, management and allocation of water. Unfortunately, the role that ICTs play to help in measuring, monitoring, and distribution of water as result of environmental issues has not been fully defined and completed.

Smart water management: meaning and objectives

Smart Water management (SWM) is a future-oriented water management strategy integrating and managing the entire process of the water cycle from analysis of current situation to purification, distribution, use and recycling of water resources scientifically and systematically. Indeed, it is an integrated management model covering the entire water cycle securing the stability, safety and efficiency of water (IBM Research, 2013). SWM can be seen as an intelligent water management model covering all aspects from water supply infrastructures to the production and distribution of water resources, digital data to manage water scientifically,. SWM seeks alleviate challenges in the water sector by promoting the coordinated development and management of water, through integration of ICT products, solutions and systems (Hope and Thomas, 2012).

Why do need smart technology for water?

Recently stakeholders in water sector are trying to incorporating ICT technology into water management extensively, pursuing smart water management to satisfy a variety of demands based on regional needs and economic conditions (Acqua, 2014). Smart technology for water management provides alternatives to the traditional solutions by applying intelligent ICT (Kappor, 2006). Today's water supply industry in faced with many imminent problems such as aging water supply infrastructure, supply and demand imbalances of water, water pollution and rising energy costs.

Smart Water Management: What it can provide with respect to conventional methods

Unlike conventional methods, SWM initiatives is not dependent on the supply capacity of water sources. It provides the optimal utilization system by combining every water resources available including underground water sea water and rain water. Therefore water can be provided and reused whenever necessary without a large scale infrastructure such as dams. Furthermore, it is to be added that SWM is capable of accurately predicting the needs and appropriate coordination of production and supply through ICT-based analysis of consumption patterns. As such, water quality and quantity and be ensured and saving can be promoted.

Smart Water Management: the tools

SWM tools fall into these main categories, data acquisition and integration (e.g. sensor, networks, smart pipes, smart meters, etc.), modelling and analytics (e.g. radio transmission, WiFi. Internet, etc., data processing and storage; (e.g. Cloud computing); management and control (eg. SCADA Optimization tools), and visualization and decision supporting (e.g. Web-based communication tools. Flow data from one end of a river without the use of stream digital geographical data can be used to create digital topography models, and videography have made it possible to store and retrieve large volumes of ground information (Mauree, 2010).

Water Smart Grid WSG

WSG is one innovative approach to addressing water scarcity, promoting efficient water use, updating existing water infrastructure, improving water quality and reducing water related energy consumption (Brozowski, 2011). The development and implementation of smart grid systems for water are similar to those that have been used for electricity distribution. A WSG

system would direct an innovative technology suite, including smart water meters, sensors, advances modelling, water mapping, smart irrigation, autonomous robots, and other technologies, that would work together to create a data-drive system for intelligent management of water resources.
These systems would be comprised of network of hardware and software that continuously monitors municipal water use ad agricultural irrigation systems; water treatment plants; individual ground water withdrawals and delivery and allocation schemes to provide consumers and utilities with real-time information on water consumption from consumer, commercial, industrial and agricultural endpoints and users (Culter, 2013).

Changing the Concepts of Water Management

The purpose of conventional water management is to ensure smooth water flow from water sources to consumers while smart water management is to transform flowing water to communicating water as it is aiming to realize water management system equipped with scientific assessment and constant communication smart water management through providing information on the whole water cycle from water source to production, supply to consumers, and re-sues (**Figure 1**) The application of the innovative smart water management approach will ensure not only stability but also both safety and efficiency of water.

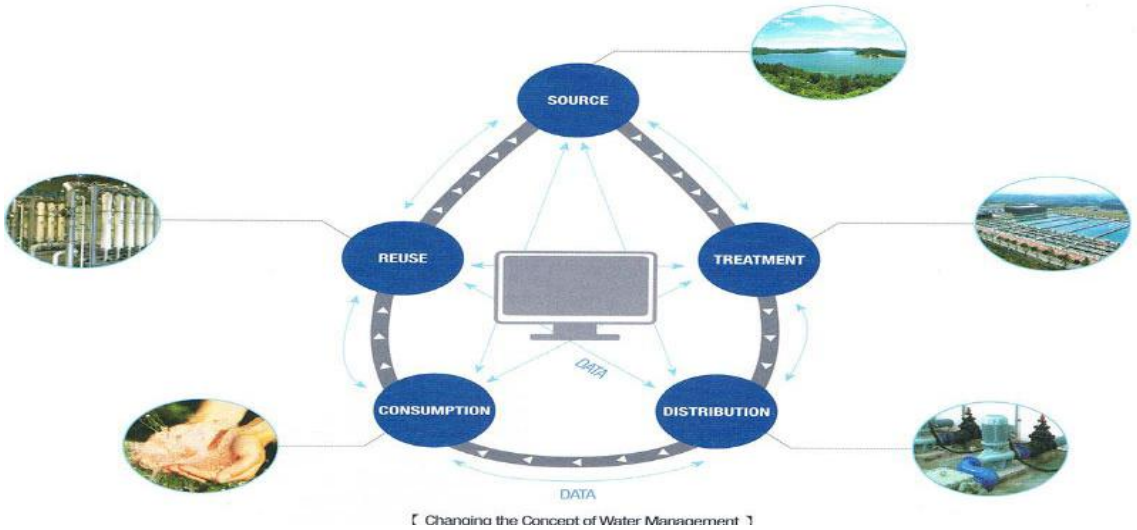

Figure 1: Changing the Concept of Water management
Source: K water , the WWF (2015)

SWM is it the water management for tomorrow management?

Smart Water consists of an information architecture and intelligent infrastructure that enable automated sensing, monitoring and decision support for water management operations. This approach includes a combination of Smart Sensors, modelling, connect and facilitate information flows. It enables continuous monitoring of physical, water infrastructure and environments, It also provides a foundation for more robust, reliable and strategic water management capabilities. With data integration, data from various sources can be combined to show the entire management domain. According to (Meena and Singht 2012), the water management projects should be based on:
- Combining science-driven conservation with IT expertise and computing power
- Building a novels software modelling framework for stimulate the behaviour of river basin around the world

- Informing policy and management decision that conserve the natural environment and benefit the people who rely on these resources. In this regard:

People technologies will be essential to successful implementation of smart system
People should be viewed as active participants of systems: sensors, decision makers , actors
People should play key role in the reflection, and reshaping of.

SWM: managing and protecting water resources. Can we implement smart Water management?

In our opinion the answer to the raised question should be not just we can, but should be we must. This is because we have the followings.
Instrumented: we now have the ability to measure, sense and see the exact condition of practically everything
Interconnected: people systems and objects can communicate and interact with each other in entirely new ways.
Intelligent: we can respond to changes quickly and accurately, and get better results by predicating and optimizing for future events.
Tackling the case of managing and protecting water resources we can think and act in more intelligent ways concerning the followings:
Eliminate data drought by improving access and delivery of the right information to drive a new era of smarter water management.
Build and repair water infrastructure whilst adding intelligence and instrumentation into the network to maximize return or investment well into the future

Water resources challenges and use of ICT

Many developing countries including those of Mediterranean are facing a series of challenges : ongoing urbanisation, resource depletion and CO_2 emission, an ageing and deteriorating urban water supply infrastructure and the effect of climate change.
Water scarcity is a real issue which will grow in importance in the near future compounded by the effect of climate change, rising population and economic growth.
To meet these challenges and to be able to drive sustainable economic growth, those countries need to tap their innovation potential through the use of Information and communication Technologies (ICTs). The use of relevant ICT and social computing can instrumentaly raise awareness of stakeholders on the significance of water sector in sustainability and in changing behaviours and attitudes among citizens. ICT can help water managers drive aggressive information campaigns and integrate the water sector with other country services in order to deliver sustainable service sand quality citizens life. The problem of future water security-including flooding and droughts is not going to go away and we must look across the whole water cycle from abstraction and treatment to use and sewage, to help address the infrastructures and attitude changes which are needed. This clearly characterize the role ICT can play in smart water management. The (OCED ,2008) in their environmental outlook to 2030 (www.oced.org) states that water demand is expected to rise globally by 55% between 2000 to 2050, by 2050 up to 3.9 billion people, 40% of the world population, may be living in water stressed area. ITCs and collaborative innovation are instrumental in helping of water management.

ICT in smart water management

In 2013, the international telecommunication Union (ITU) decided to establish a Focus Group on Smart Water Management (SWM). This initiative, aims to promote the use of ICT for

better distribution/management and allocation of water as there is lack of attention being paid to smart water management resulting in low investment level. The fact that SWM is critical to sustainable water future, nowadays the demand for intelligent and automation through ICT is increasing. The ITU has found that it is necessary for ICT to be imbedded into smart water management systems to get timely data and react to change quickly. However for smart water management, we need deeper collaboration, collective input from stakeholders to empower affected communities and to mange water and energy related challenges with an extremely high rate (Laspidou, 2014). For the energy sector ICT can enable energy network in a reasonable and sustainable way (Pagani and Aiello, 2012).

ICTs opportunities

ICT use in SMW have wide application and clear set of benefits, which, in general, increases water use efficiency and therefore decrease consumption. (Gourbesville, 2011, Martinez Liado, 2013, Savic, 2013). Some of major areas where improvement is evident could be outlined in:

Real-time monitoring: Technologies such as smart metering, SCADA, GIS, telecommunication sensors and decision support systems, all are effective tools for the provision of real-time reliable data. This means that water utilities have the opportunity to make improvements in demand response and in reducing water losses in the water distribution system;

Reduction in water consumption: ICTs provide the tools needed to create advanced water use efficiency in all sectors. Incorporation of sensors in the agricultural sectors ensures that crops are watered when needed reducing large volumes of water normally lost due to over irrigation;

Reduction in operation costs: Improvement in operational efficiencies mean that administrations costs can be reduced, creating an optimization of expenses. (Culter, 2013).

Greater public involvement: One of the benefits of ICTs is the improvement in communication between water utilities and the public which will lead to an enhancement in public awareness with respect to consumption and water usage (Alsheikh, 2011);

Integration of smart water management in smart sustainable cities (SSC): Practically, SWM is highly incorporable into SCC and it safeguards economic growth and sustain a higher quality of life as well as it maintains ecological and environmental resources for future generations (Mizuki *et al.*, 2012).

Environmental flow integration: The use of ICT products, solutions and systems in the proper monitoring and assessment of environment flow can lead to better decision-making in sustainable management of water resources (OECD, 2008)

ICTs Challenges

Although there is sustainable opportunities of ICTs use in SWM, global development in this area are currently insufficient and are mainly due to:

Lack of standardization: In spite of the efforts carried out by ITU in this field, there still need for further standardization. Standardization maintains integrity, and adherence ensures that there is compatibility, interoperability, and certain level of quality. Therefore reduction of risks (UNESCWA, 2013).

Lack of policies: Generally in most Countries there is no coherent multi-sectors and or multi-stakeholder coordinated policy on water management and /or conservation. For those Countries, it is needed to have an integrated policy formulation approach that integrates different governmental sectors, NGOs, CBOs, Academia, and the private sector for the success and adoption of water resources management policies on the national and regional level (UN-Water, 2012) .

Lack of awareness: Awareness, proper education as well dissemination are essential for proper water resources management. Many Countries are not necessary conscious on the role of ITCs can play in water management or of it is usefulness, with little attention on how ICTs can act as an enabler of SWM on large scale.
Proper ICTs governance: Lack of ICTs governance impacts investments as well as prevents stable coordinated and comprehensive planning to address future requirements and proper integration (WGF, 2012).

Key stakeholders involved in ICTs and SWM

For SWM to effectively tackles global water issues, stakeholders engagement is imperative. The type of ITCs used in SWM plan or project, is as effective and efficient, as the understanding of the roles and responsibilities played by the stakeholders. SWM stakeholders fall into diverse groups according to their roles (advising, producing solutions, making decisions.etc.). Stakeholders engagement typically involves the main areas are represented in **Figure 2**. For successful integration and implementation of SWM within countries, stakeholders involvement is necessary and should not be conducted in parallel but rather as an interactive process strategically woven throughout to determine priorities, increase support for remediation programmes and generally enhance the livelihood of success..

Figure 2. Good Stakeholders Engagement
Source: Partnering for solutions: ICTs in Smart Water Management ITU 2014

Concluding remarks and recommendations

ICT is the indispensable enabler to achieve the necessary level of control and management of water networks. What is impressive, however, is that there is no standard for data management and no data interoperability of systems across the providers, practically. Everything needs to be built. This will require a massive research effort from the ICT industry to adopt solutions to the water sector, develop adequate standards and interoperability.
One of the biggest drivers for change to smart water networks is leakage and conservation, especially in countries facing serious water scarcity issues. Leak detection is one of the most active areas of smart networking. Network monitoring, combined with analytics software, are helping to reduce the impact of leakes and identify areas in need of repair before a burst become visible and is reported.
Proper integration and stakeholders involvement prevents fragmented and uncoordinated approaches to water management issues, and therefore leads to a smarter way for water management. Appropriate intelligence adoption is SWM can only be achieved if there is focus on partnering the right technologies with the right stakeholders.

Information from utilities, municipalities, regulators, investors technology provider and academia must be bridged and are properly harnessed. This allows for the development of innovative partnership, creating the right solutions for all.

ICTs can bring enormous benefits to water authorities in mapping and monitoring of natural water resources, aquifer recharge as well as in forecasting river flows and advanced warming in water related emergency situation.

ICTs have the capability of:

Providing reliable real-time information needed for monitoring, measuring, modelling, and managing of water resources, Reliable data leads to better decision making and consequently clearer projection to cope with water scarcity and water stress problems.

To enhance water allocation by ensuring it is done in a more fair and equitable manner

To be strategic enabler to drive SMW policies and assessment.

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BIOCONCENTRATION OF LEAD IN THE POPLAR TREES (Populus robusta L.)

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Abstract

The area of the protective forest of the Glogow Copper Smelter (GCS), Poland, has been exposed to industrial emissions for many years. A large amount of dusts has been deposited in the topsoil. The paper presents results of the vulnerability assessment of poplar trees (*Populus robusta* L.) of the GCS protective forest to phytoaccumulation of lead. The bioconcentration factor (BCF), counting as the relation of Pb concentration in plant to this noted in soil, established for the poplar organs was 0.45-0.69 for leaves and 0.02-0.03 for stem. The relative small differences was observed for locations around the smelter – Zukowice I, Zukowice II and Bogomice, but very clear between them and the control site Stypulow (BCF 0.81 for leaves and 0.57 for stem). It should be taken into account absolutely different level of lead deposition in soils of the following investigation points – 175-347 $mg \cdot kg^{-1}$ in GCS protective forest against 10 $mg \cdot kg^{-1}$ in the control place. Presence of Pb in poplar leaves was about 20 times higher in the GCS protective forest comparing to the control site. There was not observed significant differences between the sites counting Pb concentration in the poplar stems. In the described situation more accurate for leaves analysis may be the average amounts described for the investigated plant species (plant pollution index – PPI).

Key words *Lead pollution, poplar tree, BCF, PPI*

Introduction

The possibility of long-term effects of emissions to the environment was the reason for the creation of the special sanitary zones around the main Polish industrial plants. An example of such a zone is the protective forest (former Glogow Sanitary Zone – GSZ) of the Glogow Copper Smelter (GCS), established in the year of 1987. The main plant species occurring on this area is Hybrid Poplar *Populus robusta* L.

For many years only the values described heavy metals concentration in soils and plants were presented. In the second half of the XX^{th} century, this situation has been changed, mainly in plant physiology and general environment elaborations. It was connected mainly with the natural values estimation for soils, waters and living organisms, including plants (Adriano 1986; Eikmann et al., 1993, Kabata-Pendias and Pendias, 2001). There were provided many researches to establish geological level of the following elements concentration in soils. As a consequence there were established the indices of state of the environment– index of geo-accumulation (Muller 1969), ecological risk index (Hakanson 1980). The next works have been focused on the threshold limits estimation and legal regulation (Eikmann and Kloke 1991, Soil and ground water criteria used in The Netherlands for contaminated land ("Dutch List") 1995, 2000, IUNG in Pulawy basis of assessment of chemical contamination of soils – Kabata-Pendias 1995, Bachmann et al. 1995, Milani et al. 1995) and evaluation of the different remediation techniques (Chaney et al. 1997, Robinson et al. 2000, Schnoor 2000, Stoltz and Greger 2002, Komárek et al. 2007, 2008).

Nowadays are discussed many problems connected with multifactorial analysis of the environment. One of the part of such analysis is the elements flow direction and intensity estimation, counting following parts of the entire environment. Thereby it became necessary to develop and apply indicators of movement of elements in soil-water, soil-plant, soil-air and others systems.
The aim of this work was to present the biosorption of Pb from the soil by the poplar plant organs – leaves and stem. Different factors were calculated to illustrate biological risk of such Pb transport in the environment.

Materials and methods

The research sites were placed in the former sanitary zone of the Glogow Copper Smelter (Lower Silesia Province, the western part of the Poland). The individual investigation sites Zukowice I (N $51^0$40’50.00” E $15^0$58’46.80”) and II (N $51^0$40’43.80” E $15^0$58’45.30”) are located about 0.1-0.3 km SW from the smelter, and Bogomice (N $51^0$41’41.60” E $16^0$00’26.50”) about 1 km NE from the smelter . The control site (Stypulow N $51^0$41’44.30” E $15^0$36’13.70”) was located about 15 km NW from the smelter.
According to Leśniczak (2005), investigations about the choice of tree species for the area were carried out from the 1973. It was tested 440 species and varieties of trees, including 287 of poplar. From the 70th of XX century the most of the area was afforested with *Populus robusta* L.Soil samplings were taken from the forest root-zone (up to 50 cm). Each sample was collected as a mixed one, representing material from the whole depth of the horizon. Soil material was air-dried and sieved with a mesh diameter of 2.0 mm.
Averaged samples of unwashed leaves of *Populus robusta* L. and stems – wood without bark, taken from saw-cut discs at a height of 1.3 m from 3 selected trees at each site. After drying, samples were ground in a mill.
Particle size distribution was determined by the Casagrande-Proszynski areometer method. Textural classes were established according to FAO procedure (2006). Sorption properties (hydrolytic acidity – HA and total exchangeable bases – TEB) were determined by the Kappen method, pH in H_2O and 1M KCl – by the potentiometric method, TOC concentration using a Shimadzu analyser, and the Pb concentration in aqua regia using atomic absorption FAAS. Extracts in Aqua regia (HCl + HNO_3 in a 3:1 ratio) were prepared according to PN-ISO 11466:2002. All analyses were performed in triplicate. All statistical analyses were conducted using Statistica 10 for Windows. The basic statistical figures were defined together with correlations between soil condition indices at levels α=0.01 and 0.05.
Contamination factors were calculated as the ratio of the Pb in plant tissues to its average soil concentration (Starck 2012) – BCF (bioaccumulation factor) and the ratio of the Pb in plant tissues to its average concentration given for analysed plant species and variety (Diatta et al. 2013) – PPI (plant pollution index).

Results and discussion

The particle size distribution of the soils covering the sanitary zone is mostly sandy loam and loamy sand. TOC concentration is not high – from 0.8% in Zukowice I locality to 1.7% in Zukowice II (Table 1). Because of this situation the soil sorption capacity reached just over 20 cmol·kg^{-1}. The soil reaction for the tested sites varies from acid in Zukowice I and Bogomice, neutral in Stypulow to alkaline in Zukowice II. Soil properties are showed in table 1.

Table 1. Selected properties of soils

SITE		Zukowice I	Zukowice II	Bogomice	Stypulow
Particle size distribution (%)					
2 mm-50 µm		78	55	53	77
50-2 µm		19	44	42	21
<2 µm		3	1	5	2
Texture class (FAO 2006)		loamy sand	sandy loam	sandy loam	loamy sand
Physic-chemical properties					
TOC (%)		0.80	1.70	1.11	0.77
pH	in H_2O	5.5	7.6	6.0	6.5
	in 1M KCl	5.0	7.2	5.5	5.5
EC ($dS \cdot m^{-1}$)		0.40	0.45	0.42	0.38
HA ($cmol \cdot kg^{-1}$)		1.38	0.21	2.47	0.66
TEB ($cmol \cdot kg^{-1}$)		10.2	12.5	25.1	14.3
CEC ($cmol \cdot kg^{-1}$)		11.5	12.5	27.7	14.9
BS (%)		87.4	98.2	89.8	94.4

TOC – organic carbon, HA – hydrolytic acidity, TEB - total exchangeable bases, CEC – cation exchange capacity, BS – base saturation

The lead concentration was similar both in soil and in plant tissues in the sites Zukowice I and Bogomice, much lower in the soil and poplar trunk of the Zukowice II (Table 2). Control site in the Stypulow locality was characterised by the low concentration of Pb in soils and in poplar leaves. The lead concentration in stem of poplar trees from the control site was higher than in Zukowice II site but smaller than in other sites.

Table 2. Pb concentration in soils and plants

SITE	Zukowice I		Zukowice II		Bogomice		Stypulow	
soil								
Minimum ($mg \cdot kg^{-1}$)	4,74		7,50		14,04		1,80	
Maximum ($mg \cdot kg^{-1}$)	328.5		175.3		347.6		9.60	
Mean value ($mg \cdot kg^{-1}$)	95,7		72,4		140,4		4,80	
Standard deviation ($mg \cdot kg^{-1}$)	37,68		30,50		58,35		1,23	
Standard error ($mg \cdot kg^{-1}$)	12,56		10,17		19,45		0,41	
plant	stem	leaves	stem	leaves	stem	leaves	stem	leaves
Minimum ($mg \cdot kg^{-1}$)	5,49	66,6	0,98	67,5	5,06	108,1	3,64	6,50
Maximum ($mg \cdot kg^{-1}$)	14,0	181,1	7,71	210,9	14,5	212,2	10,4	8,60
Mean value ($mg \cdot kg^{-1}$)	8.50	146.6	2.90	120.6	7.80	158.6	5.50	7.70
Standard deviation ($mg \cdot kg^{-1}$)	3,55	47,7	2,76	57,7	2,07	50,1	0,98	0,95
Standard error ($mg \cdot kg^{-1}$)	0,25	9,55	0,20	11,5	0,10	10,0	0,04	0,24

BCF shows very different situation (Table 3). For stem the highest result was noted for control site in Stypulow. These calculating for the GCS surrounding were lower and almost equal. The similar situation was obtained for leaves from the control site – the highest BCF values were calculated. The GCS sites were not unified, BCF in leaves for Zukowice II site was higher than these calculated for the other two sites.

PPI for stem of poplar trees was not very different for the following sites, ranging from 1.5 to 4.3. Other one situation for the leaves was noted. PPI 3.9 was characteristic for

uncontaminated control site in Stypulow locality. For the GCS surrounding PPI for leaves was between 60.3 and 79.3 (higher values for Zukowice I and Bogomice sites).

Table 3. Contamination/pollution factors for the poplar trees growing on analysed sites

SITE	stem	leaves
Bioconcentration factor (BCF)		
Zukowice I	0.03	0.45
Zukowice II	0.02	0.69
Bogomice	0.02	0.46
Stypulow	0.57	0.81
Plant pollution index (PPI)*		
Zukowice I	4.3	73.3
Zukowice II	1.5	60.3
Bogomice	3.9	79.3
Stypulow	2.8	3.9

* - as the reference value was taken 2 mg $Pb \cdot kg^{-1}$, acc. to Kabata-Pendias and Pendias (2001)

The physic-chemical condition of soils do not raise any comments about the terms of the growth and development of plants, especially for the Hybrid Poplar. *Populus robusta* L. is the tree with a wide ecological grow spectrum – soil texture: clay loams to sandy loams, soil pH 5.5-7.5 (USDAFS 1990, Seneta 2009).

Under conditions of moderate pollution of soils with heavy metals it can be observed the positive effect of mycorrhizas, limiting the heavy metal intake by plants (Christie et al. 2004). Against this, the high level of heavy metals concentration in soil can inhibit the growth of mycorrhizas (Coral del Val et al. 1999). As an effect of local conditions, the concentration of TOC in soil ranged from the 0.8 to 1.7% is described as average for Robusta stands and smaller than typical when compare with the Hybrid 275 stands (Jonczak et al. 2010).

The main problem is a purity of soils from the chemical point of view. The obtained results for areas located near the GCS ranged from 175.3 to 347.6 mg $Pb \cdot kg^{-1}$ are 18-36 times higher than this one for the control site (9.6 mg $Pb \cdot kg^{-1}$). Kabata-Pendias and Pendias (2001) described the values from 3 to 189 mg $Pb \cdot kg^{-1}$ as natural for the top horizon of different soils of the world (mean value 10-67 mg $Pb \cdot kg^{-1}$, average 32 mg $Pb \cdot kg^{-1}$). The higher values illustrate regions of the world with high developed industry and communication. Estimated Pb geometric means for soils of Poland indicate a growing Pb level with increasing concentration of clay fraction; for example, in sandy soils, 12.6 ppm; medium loamy soils, 16.4 ppm; and heavy loamy soils, 20.9 ppm (Kabata-Pendias and Pendias 2001).

As a result of industrial emissions the most vulnerable to deposition are plants and a topsoil layer. Plants absorb soil compounds by the root system. Many researchers point this part of plants as the largest store of pollutants, including heavy metals (Kabata-Pendias and Pendias, 2001; Lakhdar et al, 2010). Heavy metals absorbed by roots can be transported to the aerial parts of plants. Greszta (2002) states that in the case of poplar the concentration of lead reaches about 10 mg $\cdot kg^{-1}$ d.m. in the roots and about 33 mg kg^{-1} d.m. in branches.

The natural concentration of Pb in plants ranging from 0.1 to 10 mg $Pb \cdot kg^{-1}$ with average value 2 mg $Pb \cdot kg^{-1}$ (Kabata-Pendias and Pendias 2001). In the control site the values higher than given as average were noted, but fitted within a range (5.5-7.7 mg $Pb \cdot kg^{-1}$). Also in the GCS surrounding presence of Pb in poplar stem was not very high – 2.9-8.5 mg $Pb \cdot kg^{-1}$. Very different situation was noted for the Pb concentration in leaves – 121-159 mg $Pb \cdot kg^{-1}$. It's a typical situation for the contaminated industrial, urban and traffic areas. The most of urban areas in Poland don't reach so high Pb concentration in plant leaves. Typical levels for *Tilia*

cordata L. ranging from 7 to 32 mg Pb·kg^{-1}, for *Robinia pseudacacia* L. 4 to 31 mg Pb·kg^{-1} and for *Ligustrum vulgare* L. 3 to 20 mg Pb·kg^{-1} (Greinert 2010).

Highly significant positive correlation between the concentration of lead in the stem of trees and Pb concentration in the soil from areas of former sanitary zone has been found during the correlation analysis. There was also negative correlations between Pb in the stem and the soil reaction and organic matter concentration. The lead concentration in leaves of tested trees were highly significantly correlated with the concentration of lead in the soil, soil pH and soil clay fraction (Fig. 2). In the control area a significant correlation between the lead concentration in the leaves and stems and the Pb concentration in soil, HA and CEC has been found.

The value of accumulation factors in the analysed area varies widely – depending on compared tissues and the concentration of heavy metals in soils. The higher concentration of lead in the leaves may be due to the sedimentation of contaminants from the atmosphere. Studies conducted on *Populus alba* (Madejón et al 2004) and *Populus robusta* (Mertens et al. 2007) showed much lower concentration of lead in the leaves (about 4-5 mg·kg^{-1} d.m.) and close to the trunks of the respondents (about. 1-2 mg kg^{-1} d.m.) receiving only lead from the soil. The BCF values – comparable between areas contaminated in varying degrees, may indicate the limits of plants in the absorption of lead. This thesis study appears in Lakhdar et al. (2010).

The values of BCF for stem in contaminated sites were smaller than in control site. Additionally the values of BCF were many times smaller for stem than for leaves (Tab. 3). This observation is confirmed in studies with poplar growing in conditions of Zn, Cu, Cr and Cd soil contamination (Sebastiani et al. 2004).

Conclusion

Bioconcentration factor (BCF) was always smaller than 1, what illustrates the less of tendency to hyper accumulation of Pb by the poplar trees; BCF for leaves were higher and more different than for stem.

Plant pollution index (PPI) shows significant difference between the clean and contaminated sites, when analyse Pb concentration in leaves. For the concentration in stem PPI was found not to be a good indicator of sites contamination with Pb.

BCF is a factor which requires additional description whenever to use, because for the different situations it can show similar values.

PPI index is more accurate than BCF, but requires more detailed determination of the chemical composition of the various species, varieties, hybrids and parts (organs and tissues) of plants.

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RISK ASSESSMENT OF CHEMICAL SUBSTANCES USE TO FOREST FIRE

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Abstract

Through the implementation of the idea of sustainable development, is one of the priority objectives of the EU is (defined in the Action Plan on Sustainable Development Strategy for 2014-2020 with a strategic outlook to 2050) to ensure the society in the case of environmental care.This can be achieved by applying of appropriate tools of crisis management, which, due to the risk assessment will create space for application o protection instruments. There is necessity to continuously improve security by the systematic risk assessment of environmental and human impact. The aim of this paper is to show the risks arising from the application of extinguishing agent used by emergency services for disposal fires. The result is an overview of extinguishing agents and monitored parameters which have a negative effect of chemical substances as extinguishing agents.

Study in first part discusses the issue of forest fires that have occurred over the past decade in the Slovak Republic. Data are obtained from statistical yearbooks PTaEU Ministry of Interior of Slovak Republic and from the Statistical Office of Slovak Republic. Where as forest fires represent burning uncontrolled and undesirable negative impacts on society and the environment (including natural and cultural heritage), it should be paid to these issues still more attention. The analysis of the number and incidence of forest fires in Slovakia, the average value of forest fires of the total represents 2,5%.

The second part of study deals with observation of extinguishing by the foam which was prepared from the 3 % Sthamex foam agent water solution. The foam was applied on the Fire model A. The foam application was followed by the environmental parameters evaluation of extinguishing substance – foam, and the soil on which it was applied.

Key words: *fire, extinguishing substances, environmental impact*

Introduction

Slovakia may be a small country but with a large forest area, which represents 41% of the total country area (for forest land), which represents about 2 million hectares (Forest – natural heritage of our country is). The above data puts Slovakia among the countries with the highest rate of forestation (Kohútová, 20014). Slovak’s forests are the part of the West- Carpathian Mountains. Most forested land is in the central region of Slovakia, while 47,6% is forested area, representing almost half, followed by the East Slovak region, which has a third of its forested areas – 33,2% of the territory. Western Slovakia has the smallest forest area, around 19.2% of the territory. Listporous trees predominates the coniferous. The beech is most common tree naturally occurring in the Slovak Republic. It grows most often with fir and spruce. Such a mixing of talking trees "Carpathian mixture". Spruce in our country is represented 26.4%, while the coniferous trees are among the most common (Chromek, 2006).

The Fire and Rescue Corps (FRC) of the Slovak Republic fulfills assignments related to the fire fighting, providing the assistance and carrying out the rescue operations during

emergencies, natural disasters and other emergencies and protecting the environment, etc. Firefighters work in specific conditions. Security conditions firefighter ´s working requires a specific approach. The last decade has seen a change in the principal activity of firefighters, fire & technical interventions, as evidenced by statistics. For the purposes of evaluation and head to head comparison of exit activities of corps in Slovakia were selected data from statistical yearbooks of Fire and Rescue Corps (Official Reports, 2004-2013). For purposes to evaluate was use the statistical evaluation (descriptive statistics) in Excel. Over 10 years have been implemented 314,365 exits within the framework of FRC. The highest number of exits was in 2010 (35 654). Fig. 1 represents comparisons of individual years in terms of total number of exits of Fire and Rescue Corps compared to the number of exits to the fire. Process of the results of the total number of exits and exits to fire can be compared, except in 2004 and 2011. In 2010, the exits were to the technical intervention. That year was one of the coldest and the most raining year in Slovakia for last 140 years, which led to a significant reduction in the number of forest fires.

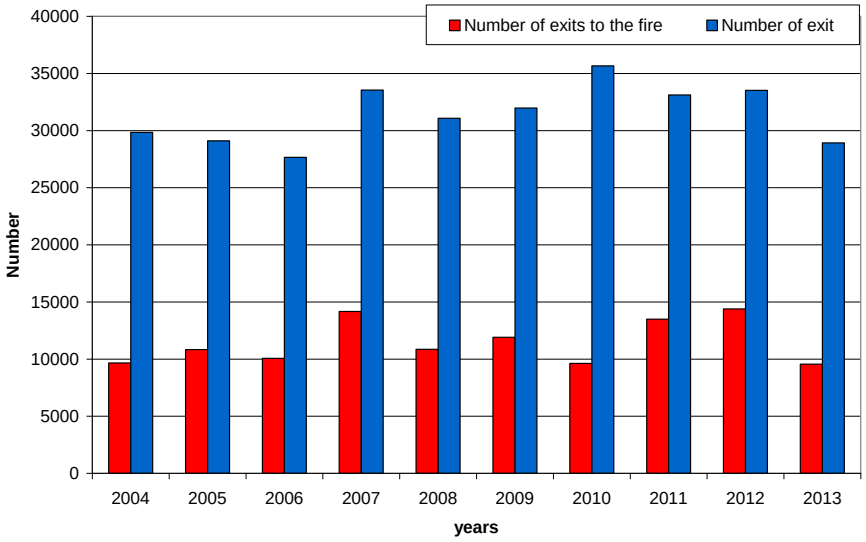

Fig. 1 Head to head comparison of the total number of exits to the number of exits Fire and Rescue Corps to the number of exits of Fire and Rescue Corps to fires from 2004 to 2013.

Forest fires on the forest ecosystem cause direct and indirect damage. Forest fires detrimental to all the components of forest biota such as habitat as well as flora and fauna (Fernandes, 2014). According to the cause of forest fires is distinguished between anthropogenic and natural harmful factors. Direct damages relate to the impairment of living trees, processed and unprocessed timber, lost growth and deterioration of wood raw material. Indirect damages related to the operation of other secondary pests, as well as the increase in the cost of the consequences of fires (Hlaváč, 2007). In the forest fire burning the whole set of organic materials that make up the forest cover. The generated heat halls in the environment affects the plants and leads to partial or complete dying back of part or the whole tree (Fernandes, 2014, Fitzgerald, 2005, Lotan et al. 1985).

Fire protection of forests against fire has to solve also the problem of used fire extinguishing substances appropriateness except the preventive precautions. The fact, that the forest fire liquidation is not easy is also confirmed by the theoretical analyses of this problem (Shaffer, 2002). Forest as well as the fires of agricultural land is extinguished by their perimeter. In previous years, the specialists from the sphere of forest fire extinguishing refer to the insufficient extinguishing efficiency of water as a extinguishing substance. (Hlaváč, 2007. Chromek, 2007). Into the water, due to the increasing if its utility parameters as well as for the fire extinguishing purposes, mainly the increasing of extinguishing efficiency of water, there

are added the additives – tensides called foam concentrates in the fire fighting practice (Marková, 2006). In the previous years, there was large market supply of the A3F Dr. Sthamex foam concentrates in our conditions. These were specialized for the extinguishing of “A” and “B” group fire, and were advised by the specialist from fire extinguishing sphere. Since the acquired results showed the excellent extinguishing parameters of these concentrates, the aim of this article is to verify the extinguishing efficiency of selected A3F foam agent on the Fire model A and to evaluate this area from the ecological aspects point of view. The above mentioned preparations show according to producer data excellent extinguishing properties, but our goal was to prove their effects on the environment after their application. *Sinapis alba* toxicity test together with two alternative ecotoxicity tests, *Thamnotoxkit* and *Daphtoxkit*, were employed for this purpose.
In order to evaluate the effectivity of extinguishing agent STHAMEX-AFFF 3% FL the experiment based on the application of extinguishing foam prepared from this agent was realized. The preparation was according to the producer data classified as dangerous chemical compound with identification as is physiologically harmless and easily biodegradable (Data sheet). Subsequently, the toxicity of the fire residuals was evaluated using three selected tests.

Material and method

The experimental ”Fire model A” is composed from wooden prisms (40 x 40 x 500 mm from spruce wood with moisture 10,5%) creating the stack, which is put on the metal frame base with the height of 250 mm, width of 900 mm and the length equal to the experimental fire model. The metal frame is made of squares of 50 x 50 mm by the ISO 657-1 standards. The scheme of the experimental ”Fire model A” is introduced in Fig. 2. According to the material economy, the fire model was constructed based on the EN ISO 14520-1 standard. For the fire ignition the flammable liquid mixture (water : fuel : kerosene in ratio 3:1:1) was used.

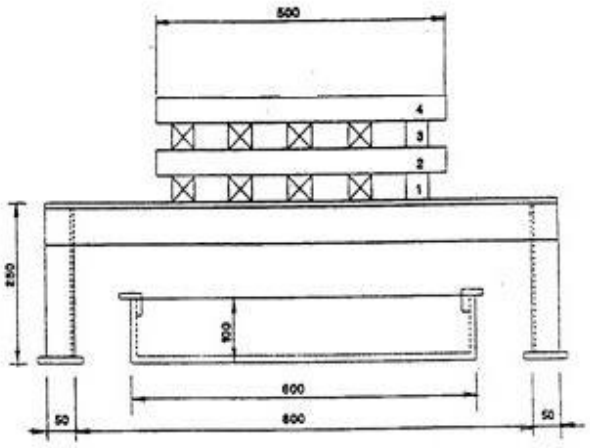

Fig. 2 Scheme of Fire model A by EN ISO 14520-1.

The extinguishing foam was produced by the use of HIRO, the water aerosol extinguishing system. From the top of the vat, there is terminated another hose, through which the compressed air flows to the branch to aerate. Owing to the STN EN 3-1 (92 0501) standards, the experiment started with the incendiary mixture lightning and parallel starting-up of a timer and the above apparatus HIRO. The Fire model A had been burning next 6 minutes that represented the total burning time of 8 minutes and creation of the experimental fire model itself. Next the fire extinguishing followed. To evaluate this experiment positively, it was important to extinguish all the fire flames not to occur repeatedly in the 3 minutes time interval of the survey. The way of extinguishing and time of fire put out was surveyed.

Extinguishing solution prepared as 3% water solution of the synthetic foaming agent STHAMEX F-15 based on surfactants was used.
For the purpose of ecotoxicological evaluation of fires and extinguishers effects artificial controlled fires were realized which were after flaring up quenched by extinguishing foam prepared from synthetic surfactants based agent STHAMEX F-15 as 3% water solution. Samples of soil and vegetation from the place after application of extinguishing foam were taken for comparison. After cooling of the fire places the samples of fire residuals were taken into the 50-liter plastic bags. All samples were transported to the ecotoxicological laboratory of the Institute of Chemistry and Technology of Environmental Protection, Brno. Ecotoxicity of extinguishing agent and samples from the fire places was evaluated using tests employing water invertebrate organism *Thamnocephalus platyurus*, which is wide-spread in our nature, and tiny crustacean *Daphnia magna*. Another applied test was the phytotoxicity test using representant of higher plants - white mustard (*Sinapis alba*). All these tests are described in details in literature Vávrová et al. (2011), Vávrová et al. (2012), Hříbová (2014).

Results and discussion

Experiments of Fire model A were especially evaluated. Among the important parameters of the measurements belonged the extinguishing time, consumption of extinguishing concentrate and the decrease of the air pressure. It follows from the obtained results that the model fire was extinguished in short time (in 9 seconds) using 4,5l of extinguishing solution. Using the 3% concentrate, the STHAMEX F-15 concentrate consumption reached the value of 0,135l. Heavy foam was prepared from the concentrate with foaming number 10,7. In other cases we concentrated on the eco-toxicological evaluation of fire places.
For the evaluation of toxicity three tests were selected. Test organisms *Daphnia magna* and *Thamnocephalus platyurus* assessed the load of the water compartment of the ecosystem when water solution of STHAMEX F-15 foaming agent was used. Another test used for the evaluation of toxicity of the STHAMEX F-15 water solution was phytotoxicity test using test organism *Sinapis alba.* Obtained results are presented in Table 1.

Table 1 Ecotoxicological evaluation for the STHAMEX 15. (Vávrová et. al., 2014)

Evaluation	Exposure	Foam Concentrate STHAMEX F-15
Daphnia magna	24h EC50	24 ml/l
Thamnocephalus platyurus	24h EC50	8 ml/l
Sinapis alba	72h IC50	32 ml/l

EC50 is concentration causing death or immobilization of 50 % of tested organisms *Daphnia magna*,
IC50 is concentration causing 50% growth inhibition of the algal culture or 50% growth inhibition of the *Sinapis alba* root in comparison with reference sample during the test duration.
Based on the performed experiments, there was tested the extinguishing efficiency of the foam, which was prepared from the STHAMEX F-15 foam agent (concentration of 3%) in the water solution. Hříbová´s (2014) study evaluated of ecotoxicological influence of the most used foam extinguishing agents concentrates (FEAs) which are often applied in fires. There were selected follows Sthamex F 15, Finiflam F 15, Expyrol F 15, Moussol APS F 15 and Pyrocool B (by effective concentration 3 – 5%). To predict ecotoxicity for aquatic ecosystems tests in aquatic arrangement using animal organisms *Thamnocephalus platyurus* and also *Daphnia magna* and plant organism *Lemna minor* and *Sinapis alba* were conducted. Their results indicated subsequently leachates of soil were contaminated by fire fighting agents via the same organisms were evaluated with aim to assess possible influence of soil sorption complex on ecotoxicity reduction. Ecotoxicological tests in contact arrangement using soil

animal organism earthworm *Eisenia fetida* and plant organism lettuce seeds *Lactuca sativa* to predict the ecotoxic effect of FEAs on terrestrial organisms and possible influence of biodegradability on ecotoxicity reduction were also conducted (Hříbová, 2014). The results of ecotoxicological tests in aquatic as well as in contact arrangements should arouse attention because of the fire fighting agents exhibit high ecotoxicity. As the least toxic agent Moussol APS F 15 was assessed, although its toxicity is still considerable (Hříbová, 2014). Our obligation is to produce chemicals that are environmentally friendly. The company aims to apply chemicals to support sustainable development. Sustainable development means providing for human needs without compromising the ability of future generations to meet their needs (Doble, Kruthiventi, 2007). The sustainable matrix, as proposed by the ICHemE (Tallis, 2002) include 49 submatrix covering the environment (related to resource usage, emissions, effluents, and waste), economy (related to profit, value, tax and investments) and society (related to workplace and society). In 2000 Young et. al. developed environmental impact factors (Tab.2) calculated using the Waste Reduction algorithm.

Table 2 Environmental factors of chemical substances

Environmental impacts	Important parameters	Several tools and methodologies are available for measuring environmental impact
Physical potential impact	ODP ozone depletion potential GWP global warming potential AP acidification potential POCP photochemical oxidation potential	LCA Life cycle assessment MIPS Material input per unit service ERA Evaluation risk assessment MFA materials low accounting CERA cumulative energy requirement analysis MFA materials flow accounting Eco Desing Life- cycle costing Total cost accounting Cost –benefit analysis
Human toxicity effects (air, water and soil)	PI human toxicity potential by ingestion TPE human toxicity potential by exposure both dermal and inhalation	
Eco – toxicity effects	ATP terrestrial toxicity potential, calculate by $ATP = \frac{1}{(LC_{50})_i}$ ATT aquatic toxicity potential calculate by $TTP = \frac{1}{(LD_{50})_i}$,	

Long-term effects of chemicals on the environment are very difficult to obtain. Thus, we have to make several assumptions and approximations while using these impact factors. This verdict relates extinguishing substances yet.

Conclusion

Based on the performed experiments, there was tested the extinguishing efficiency of the foam, from STHAMEX F-15 foam agent (concentration of 3%) in the water solution. For the evaluation of ecotoxicity only the organisms were available. From the values presented that the extinguishing agent STHAMEX F-15 doesn’t show the dangerous property H14 Ecotoxicity. Testing organism *Thamnocephalus platyurus* shows higher sensitivity to this agent. If the ecotoxicity tests were demanded using this organism, the effects of this extinguishing agent on the environment would be evaluated more strictly.

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EFFECT OF CONSERVATION PRACTICES ON SOIL MOISTURE BALANCE FOR SORGHUM YIELD UNDER RAINFED CONDITIONS OF NORTHERN INDIA

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Abstract

Field experiments were carried during rainy seasons of 2011 and 2012 in split-plot design at New Delhi, to study the impact of conservation practices on soil moisture balance for production of sorghum [*Sorghum bicolor*] under rainfed conditions. Sorghum variety ‘CSH 14’ was planted using two tillage types (minimum and conventional) and two planting methods (ridges and furrows, and flat-bed). For each type of tillage and method of planting three levels of fertilizer were applied. Soil moisture content was monitored periodically throughout the seasons by both TDR based FieldScout 300 in three soil depths (8, 12 and 20 cm), and gravimetric method for the depths (0-15, 15-30, 30-45, 45-60 and 0-60 cm) to study the soil moisture retention in each treatment. Soil moisture balance was determined periodically throughout the crop growing season. The results showed that the ridges and furrows planting method in conventional tillage retained the highest soil moisture content leading to the highest grain yield of 2.03 and fodder yield of 9.42 tonnes/ha. Flatbed sowing in minimum tillage was found to retain the least soil moisture content. The ranking of treatments in grain and fodder yields were similar to the ranking of retention of soil moisture content. The increment and decrement of the soil moisture balance was according to the effective rainfall and the total crop evapotranspiration (ETc) during normal rainfall season. The study suggested adoption of ridges and furrows method of planting in conventional tillage with 100% recommended dose of fertilizer for sorghum production as the one of best conservation technology.

Keywords*:* *Sorghum; Ridges and furrows; Conventional tillage; Soil moisture balance*

Introduction

The soil water balance is widely assumed to be the primary factor limiting crop yield. Water shortage and stress were the most important abiotic constraint to effect crop production globally and a major cause of yield instability and food insecurity. However, rain-fed agriculture is not coping for unreliable rainfall and recurrent droughts that leads to subsequent production failures (Zougmore *et al.* 2002). Low soil fertility and erratic rainfall are responsible for the low agricultural productivity of rainfed agriculture in African continent and India (Anonymous 2000). Climate change scenarios indicate that water shortage and shortening of the effective growing season will be increasing the need for short-duration cereals such as sorghum which is widely grown under rain-fed conditions in drought-prone regions of Africa and Asia. Sorghum (*Sorghum bicolor* Moench) is a drought tolerant cereal and has evolved various ecotypes that withstand an array of biotic and abiotic stresses (Elbasri *et al.* 2011). Sorghum should not be planted in a dryland situation unless the soil has stored moisture to a minimum depth of one meter (Jennie *et al.* 2005). Phil *et al.* (2004) indicated that moisture stress at any time after planting can affect plant development and reduce yield potential. The amount of yield loss is dependent on the growth stage of the grain sorghum when moisture stress occurs. Therefore, application of suitable soil and water management practices such as tied-ridging tillage should be integrated with fertilizer to improve the soil

water and soil fertility at the same time for production enhancement (Gebreyesus, 2012). Planting of crops can be either in the furrow or on the ridge based on the expected soil moisture required for a particular crop (Gebreyesus *et al.,* 2006; Gebreyesus and Wortmann, 2008; Tewodros *et al.,* 2010). Soil moisture accounting is used to calculate the soil-water balance in the root zone throughout the growing season (Phil *et al*. 2004). Despite the above facts, however, so far, there have been insufficient published results that estimate the role of ridges and furrows and other tillage practices as part of soil moisture harvesting technique integrated with fertilizer at small fields using hybrid varieties of sorghum. Thus, the aim of this research was designed to evaluate the effect of tillage practices on soil moisture balance and effect of retention of soil moisture and inorganic fertilizer concurrently on the productivity of CSH 14 variety of sorghum at small scale cultivation for precise evaluation.

Materials and Methods

The field experiment was conducted during rainy seasons of 2011 and 2012 at Water Technology Centre, Indian Agricultural Research Institute, New Delhi, (28° 38' 23" N of 77° 09' 27" E, at an average elevation of 228.61 m above the mean sea-level). The site has semi-arid and subtropical climate with hot dry summer and severe cold winter. Average annual rainfall is about 650 mm, 75% of which is received in July, August and September. The experiment was laid out in 3 replications in split-plot design for sorghum variety ‘CSH 14’. The treatments consisted of 2 types of tillage, viz. minimum and conventional, in main plots, 2 methods of planting, viz. ridges and furrows and flatbed, in sub-plots, and 3 levels of fertilizer. The full recommended dose of fertilizer (100%RDF) was 50 kg/ha of urea (46% nitrogen), 25 kg/ha single superphosphate (16% P_2O_5) and 25 kg/ha muriate of potash (60% K_2O). Sowing was done manually by labourers for the both seasons on 24 June 2011 and 11 July 2012. The delayed onset of autumn was the main reason of the delay in seeding process in the second year. Meteorological data, periodic volumetric soil moisture content for the soil depths 8, 12 and 20 cm using Time Domain Reflectometry (TDR) model FieldScout TDR 300 and gravimetric soil moisture content, were recorded throughout the growing season. Soil samples were taken from 0-15, 15-30, 30-45, 45-60 and 0-60 cm soil depth by adopting recommended sampling techniques and analyzed for gravimetric soil moisture content. The total rainfall received during the sorghum growing seasons of 2011 and 2012 were 574.0 mm and 483.2 mm respectively. The effective rainfall was estimated by fixed percentage approach, which is an empirical formula developed by Food and Agriculture Organization of the United Nations (FAO) and Agricultural, Land and Water Division (AGLW) based on analysis carried out for different climatic data to determine the dependable effective rainfall, i.e., the dependable rainfall at 80% probability corrected for assumed losses due to runoff and percolation (Smith, 1988). Reference evapotranspiration (ETo) and the crop water demand (ETc) were estimated using climate and soil data with CROPWAT 8.0 software (FAO, 2008). The soil moisture content on dry weight basis was determined using the following equation:

$$Soil\ moisture\ content\ (\%) = \frac{Weight\ of\ Wet\ soil\ (g) - Weight\ of\ Dry\ soil\ (g)}{Weight\ of\ Dry\ soil\ (g)} \times 100$$

Conversion of soil moisture content (%) to mm of moisture per m of soil was done by the following expression (Ratliff *et al.* 1983**):**

$$Soil\ moisture\ content\ (mm/m) = Soil\ moisture\ content\ (\%) \times \left[\left(\frac{1000\ (mm)}{1\ (m)}\right) \div 100\right]$$

The effective root zone was assumed as 1.20 m. Data on sorghum crop coefficients (K_C) are provided by the software (Some L. *et al.* (2006); FAO, 2008; R. Balaghi, 2010; Anonymous, 2010). Water balance was estimated by:

$$Soil\ Water\ balance = Ef\ rainfall - ETc$$

The data on volumetric and gravimetric soil moisture content were analyzed statistically by using analysis of variance according to Gomez and Gomez (1984) procedure for a split-plot design (3 factors) and the means separation were identified by least significant differences (L.S.D) at $P \geq 0.05$, by using MSTAT-C Package, version 1.47.

Results and Discussion

Soil moisture conservation

From Tables 1, 2, 3 and 4 the results revealed that there were significant differences in volumetric and gravimetric soil moisture content (at 5% level of significance) of the both seasons between the treatment of the ridges and furrows sowing in conventional tillage (T_{21}) and with each one of the other treatments for the all soil depths. The differences were highly significant between the ridges and furrows sowing in conventional tillage and flatbed sowing in minimum tillage. These results confirmed that the ridges and furrows sowing in conventional tillage have a significant impact on the ability of the soil to conserve moisture, followed by ridges and furrows sowing in minimum tillage (T11), flatbed sowing in conventional tillage (T22) and flatbed sowing in minimum tillage (T12) respectively.

Table 1. Mean differences and significance level for volumetric soil moisture content, for crop growing season of the year 2011

Differences between treatments	Soil depth (cm)	Observation date									Grand Mean
		24/6	30/6	4/7	28/7	14/8	30/8	12/9	7/10	25/10	
Difference between T_{21} and T_{11}	8	2.1^{S}	2.4^{S}	1.4^{NS}	1.6^{S}	0.1^{NS}	0.4^{NS}	0.5^{NS}	2.7^{*S}	2.6^{*S}	1.5^{S}
	12	2.2^{S}	1.6^{S}	0.1^{NS}	2.5^{*S}	0.6^{NS}	1.2^{NS}	0.2^{NS}	3.3^{*S}	3^{*S}	1.6^{S}
	20	0.8^{NS}	2.3^{S}	0.2^{NS}	1.3^{NS}	1.1^{NS}	0.5^{NS}	0.3^{NS}	1.7^{S}	2^{S}	1.1^{S}
Difference between T_{21} and T_{12}	8	2.9^{*S}	3.8^{**S}	2.9^{*S}	3^{*S}	2.4^{S}	2^{S}	0.6^{NS}	5.8^{**S}	5.5^{**S}	3.2^{*S}
	12	3.8^{**S}	4.8^{**S}	2.3^{S}	3.5^{**S}	1.6^{S}	2.2^{S}	1.2^{NS}	5.8^{**S}	6^{**S}	3.5^{**S}
	20	4.2^{**S}	2.7^{*S}	0.2^{NS}	1.8^{S}	0.6^{NS}	0.8^{NS}	0.5^{NS}	4.9^{**S}	4.6^{**S}	2.3^{S}
Difference between T_{21} and T_{22}	8	1.7^{S}	2.3^{S}	2.1^{*S}	1.8^{S}	1.8^{S}	2.1^{*S}	0.9^{NS}	4.7^{**S}	3.3^{*S}	2.3^{S}
	12	2.2^{S}	0.7^{NS}	1.4^{NS}	0.8^{NS}	1.4^{NS}	2.1^{*S}	1.1^{NS}	4.2^{**S}	3.9^{**S}	2.0^{S}
	20	1.9^{S}	1^{NS}	0.7^{NS}	1.3^{NS}	1.6^{S}	1.8^{S}	1.9^{S}	4^{**S}	3^{*S}	1.9^{S}
Grand Mean		2.4^{S}	2.4^{S}	$1.3N^{S}$	2.0^{S}	1.2^{NS}	1.5^{S}	$0.8N^{S}$	4.1^{**S}	3.8^{**S}	2.2^{S}
LSD value		1.23	1.78	1.78	1.31	1.29	1.08	0.68	1.00	0.45	

Note: NS: Non Significant ranged 0.1 – 1.4, S: Significant ranged 1.5 - 2.4, *S: High Significant ranged: 2.5 – 3.4 and **S: Highly Significant > 3.5

Table 2. Mean differences and significance level for volumetric soil moisture content, for crop growing season of the year 2012

Differences between treatments	Soil depth (cm)	Observation date									Grand Mean
		9/7	16/7	28/7	5/8	19/8	31/8	12/9	30/9	15/10	
Difference between T_{21} and T_{11}	8	2.1^{S}	2.1^{S}	2.1^{S}	1.9^{S}	0.6^{NS}	0.6^{NS}	1^{NS}	2.6^{*S}	2.3^{S}	1.7^{S}
	12	2.2^{S}	2.1^{S}	2.1^{S}	1.8^{S}	1.4^{S}	1.4^{S}	1.5^{S}	3^{*S}	2.6^{*S}	2.0^{S}
	20	0.8^{NS}	0.8^{NS}	0.8^{NS}	1.1^{NS}	0.2^{NS}	0.2^{NS}	0.7^{NS}	2^{S}	1.4^{S}	0.9^{NS}
Difference between T_{21} and T_{12}	8	2.9^{*S}	3^{*S}	3^{*S}	3^{*S}	1.6^{S}	1.6^{S}	2.9^{*S}	5.5^{**S}	4.2^{**S}	3.1^{*S}
	12	3.8^{**S}	3.8^{**S}	3.8^{**S}	3.7^{**S}	2.8^{*S}	2.9^{*S}	3.5^{**S}	6^{**S}	4.5^{**S}	3.9^{**S}
	20	4.2^{**S}	4.2^{**S}	4.2^{**S}	3^{*S}	1^{NS}	1^{NS}	2.5^{*S}	4.6^{**S}	4.4^{**S}	3.2^{*S}
Difference between T_{21} and T_{22}	8	1.7^{S}	1.7^{S}	1.8^{S}	1.8^{S}	2.2^{S}	2.2^{S}	2.8^{*S}	3.3^{*S}	2.5^{*S}	2.2^{S}
	12	2.1^{S}	2^{S}	2^{S}	1.4^{S}	2.9^{*S}	2.9^{*S}	2.6^{*S}	3.9^{**S}	3.9^{**S}	2.6^{*S}
	20	1.9^{S}	1.9^{S}	1.9^{S}	1.6^{S}	2.1^{S}	2.1^{S}	2.8^{*S}	3.1^{*S}	2.5^{*S}	2.2^{S}
Grand Mean		2.4^{S}	2.4^{S}	2.4^{S}	2.1^{S}	1.6^{S}	1.7^{S}	2.3^{S}	3.8^{**S}	3.1^{*S}	2.4^{S}
LSD value		1.23	1.23	1.23	1.06	1.14	1.14	0.75	0.47	0.89	

Note: NS: 0.1 – 1.3, S: 1.4 - 2.4, S*: 2.5 – 3.4 and S**: > 3.5

Table 3. Mean differences and significance level for gravimetric soil moisture content, for crop growing season of the year 2011

Differences between treatments	Soil depth (cm)	Observation date									Grand Mean
		24/6	30/6	4/7	12/7	16/8	31/8	12/9	10/10	20/10	
Difference between T_{21} and T_{11}	0-15	1.7^{S}	1.9^{S}	1.6^{S}	1.3^{S}	0.8^{NS}	1.2^{S}	0.3^{NS}	2.1^{S}	2.2^{S}	1.5^{S}
	15-30	1.5^{S}	1.2^{S}	0.3^{NS}	1.2^{S}	0.5^{NS}	1.3^{S}	0.5^{NS}	1.8^{S}	2^{S}	1.1^{S}
	30-45	1.2^{S}	2.3^{S}	0.7^{NS}	1^{S}	0.3^{NS}	1.3^{S}	0.7^{NS}	2^{S}	1.9^{S}	1.3^{S}
	45-60	1.1^{S}	3.9^{**S}	1.1^{S}	1.5^{S}	0.6^{NS}	1^{S}	0.7^{NS}	2.7^{*S}	1.8^{S}	1.6^{S}
	0-60	2.2^{S}	2.8^{*S}	1.5^{S}	1^{S}	0.7^{NS}	1.1^{S}	0.9^{NS}	2.4^{S}	1.8^{S}	1.6^{S}
Difference between T_{21} and T_{12}	0-15	2.3^{S}	2.9^{*S}	2.5^{*S}	2.1^{S}	2.2^{S}	1.9^{S}	0.7^{NS}	4.3^{**S}	4.1^{**S}	2.6^{*S}
	15-30	2.5^{*S}	3.2^{*S}	1.7^{S}	1.7^{S}	1.3^{S}	2.2^{S}	1.1^{S}	3.7^{**S}	3.8^{**S}	2.4^{S}
	30-45	3.4^{**S}	2.5^{*S}	0.9^{NS}	1.5^{S}	0.8^{NS}	1.8^{S}	1.1^{S}	4^{**S}	3.6^{**S}	2.2^{S}
	45-60	3.3^{**S}	2.8^{*S}	2.8^{*S}	2^{S}	1^{S}	1.5^{S}	1.1^{S}	4.1^{**S}	3.5^{**S}	2.5^{*S}
	0-60	2.4^{S}	3.6^{**S}	2.2^{S}	1.4^{S}	1.5^{S}	1.5^{S}	0.8^{NS}	3.7^{**S}	3.4^{**S}	2.3^{S}
Difference between T_{21} and T_{22}	0-15	1.2^{S}	1.6^{S}	1.5^{S}	0.9^{NS}	1.1^{S}	1.6^{S}	0.9^{NS}	3.2^{*S}	2.2^{S}	1.6^{S}
	15-30	1.3^{S}	0.5^{NS}	0.9^{NS}	0.6^{NS}	0.8^{NS}	1.9^{S}	0.7^{NS}	2.6^{*S}	2.3^{S}	1.3^{S}
	30-45	1.4^{S}	0.7^{NS}	0.4^{NS}	1^{S}	0.3^{NS}	1.5^{S}	1.1^{S}	2.9^{*S}	2.1^{S}	1.3^{S}
	45-60	1.4^{S}	1.7^{S}	1.1^{S}	1.3^{S}	0.3^{NS}	0.8^{NS}	1.2^{S}	2.8^{*S}	1.9^{S}	1.4^{S}
	0-60	0.6^{NS}	1.3^{S}	1.5^{S}	0.8^{NS}	0.9^{NS}	1^{S}	1.1^{S}	2.4^{S}	1.8^{S}	1.3^{S}
Grand Mean		1.8^{S}	2.2^{S}	1.4^{S}	1.3^{S}	0.9^{NS}	1.4^{S}	0.9^{NS}	3.0^{*S}	2.6^{*S}	1.7^{S}
LSD value		1.00	1.33	1.19	0.75	0.82	0.78	0.48	0.67	0.34	

Note: NS: 0.1 – 0.9, S: 1- 2.4, *S: 2.5 – 3.4 and **S: > 3.5.

Table 4. Mean differences and significance level for gravimetric soil moisture content, for the crop growing season for the year 2012

Differences between treatments	Soil depth (cm)	Observation date									Grand Mean
		9/7	16/7	5/8	12/8	19/8	31/8	12/9	23/9	15/10	
Difference between T_{21} and T_{11}	0-15	1.7^{S}	1.7^{S}	1.6^{S}	1.2^{S}	1^{S}	1.1^{S}	1.1^{S}	2^{S}	1.9^{S}	1.5^{S}
	15-30	1.6^{S}	1.6^{S}	1.5^{S}	3.3^{*S}	1.4^{S}	1.5^{S}	1.3^{S}	2^{S}	1.8^{S}	1.8^{S}
	30-45	0.9^{S}	1.1^{S}	1.2^{S}	3^{*S}	0.8^{NS}	0.9^{S}	1.1^{S}	1.7^{S}	1.3^{S}	1.3^{S}
	45-60	1.2^{S}	1.2^{S}	1.5^{S}	0.6^{NS}	1.2^{S}	1.2^{S}	1.3^{S}	2^{S}	1.6^{S}	1.3^{S}
	0-60	1.5^{S}	1.4^{S}	1.6^{S}	1.2^{S}	1.3^{S}	1.3^{S}	1.5^{S}	2^{S}	1.8^{S}	1.5^{S}
Difference between T_{21} and T_{12}	0-15	2.2^{S}	2.2^{S}	2.3^{S}	2.2^{S}	1.6^{S}	1.7^{S}	2.4^{S}	4.2^{**S}	3.1^{*S}	2.4^{S}
	15-30	2.5^{*S}	2.6^{*S}	2.6^{*S}	5.8^{**S}	2.4^{S}	2.3^{S}	2.5^{*S}	3.9^{**S}	3.2^{*S}	3.1^{S}
	30-45	3.1^{*S}	3.2^{*S}	2.5^{*S}	2.8^{*S}	1.4^{S}	1.5^{S}	2.3^{S}	3.7^{**S}	3.3^{*S}	2.6^{*S}
	45-60	2.7^{*S}	3^{*S}	2.8^{*S}	3.7^{**S}	1.8^{S}	1.8^{S}	2.6^{*S}	4^{**S}	3.6^{**S}	2.9^{*S}
	0-60	2.8^{*S}	3^{*S}	2.7^{*S}	2.8^{*S}	1.8^{S}	1.9^{S}	2.6^{*S}	4^{**S}	3.6^{**S}	2.8^{*S}
Difference between T_{21} and T_{22}	0-15	1.2^{S}	1.2^{S}	1.2^{S}	0.1^{NS}	1.5^{S}	1.6^{S}	1.9^{S}	3.1^{*S}	1.7^{S}	1.5^{S}
	15-30	1.3^{S}	1.3^{S}	0.9^{S}	1.4^{S}	1.8^{S}	1.8^{S}	1.7^{S}	2.6^{*S}	1.8^{S}	1.6^{S}
	30-45	1.3^{S}	1.4^{S}	1^{S}	1.8^{S}	1.4^{S}	1.4^{S}	1.9^{S}	2.7^{*S}	1.7^{S}	1.6^{S}
	45-60	1.3^{S}	1.1^{S}	1.1^{S}	0.1^{NS}	1.4^{S}	1.4^{S}	1.9^{S}	2.8^{*S}	1.8^{S}	1.4^{S}
	0-60	1.4^{S}	1^{S}	1.2^{S}	0.3^{NS}	1.4^{S}	1.4^{S}	1.9^{S}	2.7^{*S}	1.8^{S}	1.5^{S}
Grand Mean		1.8^{S}	1.8^{S}	1.7^{S}	2.0^{S}	1.5^{S}	1.5^{S}	1.9^{S}	2.9^{*S}	2.3^{S}	1.9^{S}
LSD value		0.83	0.87	0.71	1.36	0.73	0.74	0.52	0.67	0.59	

Note: NS: 0.1 – 0.8, S: 0.9- 2.4, *S: 2.5 – 3.4 and **S: > 3.5

These results are in conformity with the finding of Memon, 2007 who reported that soil moisture content after tillage operation showed highly significant difference in all the treatments and it was conserved more in ridge plot as compared to other treatments.

Soil water balance

Soil moisture monitoring for determination of soil moisture retention and soil water balance was taken for 60 cm soil depth. Positive value of soil moisture balance indicate that the effective rainfall is adequate to meet the crop water demand and the negative value indicate that the effective rainfall is inadequate to meet the crop water demand and supplementary

irrigation was needed. The total water balance of 73.3 and 155.7 (Table 5 and 6) respectively for the both season as per growing stages, should be supplemented by precise and efficient supplementary irrigation to achieve optimum yield under these conditions of tillage types and planting methods treatments with 100% RDF.

Tables 5. Soil water balance, effective rainfall, ETc of sorghum crop and observed soil moisture retention in 60 cm soil depth and for season of the year 2011

Month	Stages of the crop growth	Eff rainfall (mm/dec)	ETc (mm/dec)	Soil water balance (mm/dec)	Observed soil moisture retention (mm)			
					T_{11}	T_{12}	T_{21}	T_{22}
June	Initial	15.1	10.1	(+) 5	48.8	37.6	52.1	39.0
July	Initial	0	12.6	(-) 12.6	55.8	42.3	63.5	47.0
July	Development	12.5	12.5	(+) 0.0	61.2	56.8	70.4	61.1
July	Development	33.6	19.7	(+) 13.9	72.0	68.2	76.5	71.0
August	Development	70.2	23.7	(+) 46.5	87.9	83.7	92.6	85.1
August	Development	85.8	28.6	(+) 57.2	88.0	85.3	94.0	86.8
August	Mid	78.8	36.8	(+) 42.0	77.7	76.1	82.7	77.1
September	Mid	64.7	35	(+) 29.7	49.2	41.7	63.8	49.0
September	Mid	55.7	36.4	(+) 19.3	47.6	37.5	58.2	47.3
September	Late	42.8	35.1	(+) 7.7	46.0	36.2	53.8	45.2
October	Late	0	31.6	(-) 31.6	45.8	42.3	51.5	43.0
October	Late	0	27.1	(-) 27.1	43.8	37.6	50.1	39.0
October	Late	0	2.0	(-) 2.0	42.1	32.3	49.9	36.3
Total		459.2	311.2					

Tables 6. Soil water balance, effective rainfall, ETc of sorghum crop and observed soil moisture retention in 60 cm soil depth and for season of the year 2012

Month	Stages of the crop growth	Eff rainfall (mm/dec)	ETc (mm/dec)	Soil water balance (mm/dec)	Observed soil moisture retention (mm)			
					T_{11}	T_{12}	T_{21}	T_{22}
July	Initial	42.6	14.3	(+) 28.3	46.5	33.6	53.3	43.7
July	Development	54.4	14.2	(+) 40.2	52.9	40.0	58.4	51.6
August	Development	76.5	15	(+) 61.5	63.0	44.2	67.5	53.6
August	Development	67.7	17.4	(+) 50.3	66.9	46.4	68.2	48.6
August	Development	87.6	27.7	(+) 59.9	84.9	81.7	90.7	82.5
September	Mid	36.5	35.4	(+) 1.1	77.9	70.1	83.9	75.8
September	Mid	12.2	39.8	(-) 27.6	56.3	49.4	59.5	52.4
September	Mid	9.1	37.7	(-) 28.6	54.0	41.8	58.6	48.5
October	Late	0	35.7	(-) 35.7	45.9	35.9	56.8	44.8
October	Late	0	30.6	(-) 30.6	45.6	35.1	55.2	45.3
October	Late	0	23.7	(-) 23.7	45.2	34.1	56.3	44.4
November	Late	0	9.5	(-) 9.5	43.0	33.3	49.7	41.2
Total		386.6	301.0					

These results concluded that the increment and decrement of the soil moisture content naturally balancing according to the effective rainfall and the total ETc, when the rainy season is normal. The results are in close conformity with the finding of Some L. *et al.* (2006), which stated that soil water reserves naturally increase according to rainfall which generally decrease at the onset of the rainy season and sharply increase during the period of heavy rains to reach a maximum value in early September in regions of Bobo and Gaoua in Burkina Faso. Generally the values of water avilable in the soil for the crop were higher in the first season than the second one. The rainfall distribution in the first season was better than the second season leading to better crop production in the first season as compared to second season. The water stress in the end of this season did not affect the yield, because the crop was at the last stage of panicles and grain drying and did not need much water (Table 5). But the water stress

in the end of the second season was caused some yield reduction, because the crop was at the begining of the late stage of the grain filling process which required water to proceed normally (Table 6). The ridges and furrows method of planting in conventional tillage with 100% RDF recorded the highest grain yield of 2.03 and fodder yield of 9.42 tonnes/ha, and the ranking of treatments in grain and fodder yields similar to their ranking in soil moisture retention(Gabir et al., 2014).

Conclusions

The results of the present study concluded that, Adoption of conservation tillage practices for sorghum production proved optimum for higher soil moisture retention in the two growing seasons. Amongst the different planting systems, ridges and furrows in conventional tillage for the two growing seasons gave the highest soil moisture retention which constituted 32.5% more of the soil moisture retention in flatbed planting in minimum tillage. Adoption of ridges and furrows planting in conventional tillage with 100% recommended dose of fertilizer led to higher soil moisture retention and higher yield of 55% more as compare to flatbed planting in minimum tillage. The increment and decrement of the soil moisture content naturally balancing according to the effective rainfall and the total crop evapotranspiration under normal rainy season. Sorghum did not require supplementary irrigation under normal rainy season. The water deficit at grain filling stage should be supplemented by precise and efficient supplementary irrigation to prevent reduction in yield.

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EFFECTS OF CHLORPYRIFOS AND GLYPHOSATE PESTICIDES ON BEHAVIOUR OF EARTHWORM IN THE SOIL

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Abstract

Pesticides are important element of modern agriculture. Although pesticides are designed to kill harmful organisms, their environmental risks are important, especially soil applicated ones. Small amount of pesticides is reached to targeted organisms, rest is still in the ecosystem. Adverse effect to bees, birds, fish and microorganisms are also important issue.

Earthworms provide key soil functions that favour many positive ecosystem services. They represent a large fraction of soil living biomass in many temperate ecosystems and play an important role in soil functioning. As ecosystem engineers they influence organic matter dynamics, soil structure and microbial community. They participate in soil aeration, water infiltration and mixture of soil horizons, and they represent an important source of food for many other organisms like birds. These services are important for agro ecosystem sustainability, but can be degraded by intensive use of pesticides.

In this original research, we aimed to investigate the effect of two pesticides on behaviour of earthworms. For this aim chlorpyrifos and glyphosate treated soil was placed to the 2D (2 Dimensions) glass apparatus (Din–A4/ 21.0 x 29.7 x 0.4 cm). Then earthworms were released to the top of apparatus. Application rates were recommended dose and its two fold. Tunnel (gallery) opening performance and other behaviour of worms compared in the control and treated ones. Tunnel areas measured by using Sketchup program, then the results evaluated statistically. According the our findings there is no statistically significant diffrences between treated (recommended dose and twice) and control experiment.

Key Words: *Pesticide, environmental, risk, earthworms*

Introduction

Although pesticides are designed to kill harmful organisms, their environmental risks are important, especially soil applicated ones. Small amount of pesticides is reached to targeted organisms, rest is still in the agro ecosystem (Tiryaki and Temur, 2010). Use of pesticides often result in a loss of biodiversity. Their adverse effect to bees, birds, fish and microorganisms are also important issue. The effects of pesticides on different taxa and especially on soil organisms are not very clear (Pelosi et al., 2014).

Earthworms provide key soil functions that favour many positive ecosystem services. They represent a large fraction of soil living biomass in many temperate ecosystems and play an important role in soil functioning. Because of the major role, they play in soil functioning, the effects of pesticides on these soil organisms should be investigated further. As ecosystem engineers they influence organic matter dynamics, soil structure and microbial community. They participate in soil aeration, water infiltration and mixture of soil horizons, and they represent a source of food for many other organisms. These services are important for ecosystem sustainability, but can be degraded by intensive use of pesticide (Pelosi et al., 2014)

It is underlined the importance of earthworms for assessing the general impact of pollution in soil. Since then, earthworms have sometimes been used as bio indicators for soil

quality and the environmental impacts of cropping systems and pollutants (Callahan, 1984). Since then, earthworms have sometimes been used as bio indicators for soil quality and the environmental impacts of cropping systems and pollutants. Earthworms are impacted by pesticides at all organisation levels, such as the infra-individual level, the individual and population levels and the community level. For example, pesticides disrupt enzymatic activities, increase individual mortality, decrease fecundity and growth, change individual behaviour such as feding rate and decrease the overall community biomass and density (Pelosi et al., 2014). For instance, mortality and/or reproduction of *Eisenia fetida* are currently used to assess the effects of pesticides under laboratory conditions before marketing authorisation (ISO, 1998). Capowiez et al. (2010) worked on earthworm behaviour as a biomarker**.** Casabé et al. (2007) investigated the effect of chlorpyrifos and glyphosate on earthworm.

The aim of this original research is to investigate the effect of 2 pesticides on behaviour of earthworms (*Eisenia foetida).* For this aim commercial formulation of them used in this study. Both pesticides mostly used as soil applicated insecticide and herbicide in Turkey.

Materials and Methods

Materials

The earthworms known as California earthworm *Eisenia foetida,* are obtained from Siyah Altın Compnay- Edirne, Turkey. Sandy-loam soil is used in this study. Further properties of soil are shown in Table 1. Pestban 25 WP (commercial formulation of chlorpyrifos active ingredient from Organophosphate group) and Boxer (commercial formulationt of glypohosate active ingredient from Phosphonoglycine group) are used in the experiment. Detaily properties of both pesticides are given in Table 2. Other basic laboratory glass equipment and solvent were used in the study.

Table 1. Properties of soil used in the experiment

Properties	Value	Review
pH (1:2.5)	8.1	Mild Alkali
Sand (%)	56	Sandy loam
Clay(%)	16	
Shaft (%)	28	
Organic Matter (%)	3.8	Adequate Values
N, ppm	0.66	Rich
P, ppm (Olsen)	7	20-25
K, ppm	150	200-320
Ca, ppm	2813	1440-6120
Mg, ppm	287	117-400
Fe, ppm	7.5	2.5-4.5
Mn, ppm	14.1	>1
Zn, ppm	0.9	>1
Cu, ppm	0.7	>0.2

Experimenteal procedure

2D (2 Dimentions, Din–A4/) apparatus was constructed with 21.0 x 29.7 x 0.4 cm diamentions glass. 4 mm wooden rods were put between 2 glass plate, and small cloth were placed bottom of the apparate to absorb water, then apparatus was fixed with 4 clamp with 4 corner (Türkmen et al, 2013). Boxer 48 SL herbicide and Pestban 25 WP insecticide were

Table 2. General, toxicological, ecotoxicological and Environmental properties of pesticide active ingredients (FOOTPRINT, 2015).

Property	Chlorpyrifos	Glyphosate
Pesticide type	Insecticide	Herbicide
Substance origin	Synthetic	Synthetic
Mode of action	Acetylcholinesterase(AChE) inhibitor.	Inhibition of EPSP synthase.
Chemical formula	$C_9H_{11}Cl_3NO_3PS$	$C_3H_8NO_5P$
Molecular mass (g /mol)	350.89	169.1
IUPAC name	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate	N-(phosphonomethyl)glycine
ADI-Acceptable Daily Intake (mg/kg bw/day)	0.001	0.30
Mammals-Acute oral LD_{50} (mg/kg)	64.0	>2000
Mammals-Dermal LD_{50} (mg/kg vücut ağ.)	>1250	>2000
Mammals-Inhalation LC_{50} (mg/l)	0.10	0.10
Earthworms-Acute 14 day LC50 (mg/kg) *Eisenia foetida*	129.0	>480
Earthworms-Chronic 14 day NOEC, reproduction (mg/kg) *Eisenia foetida*	12.70	>28.8
Solubility In water at 20°C (m/l)	1.05	10500
Henry's law constant at 25°C (Pa m^3/mol)	0.478	2.10×10^{-07}
GUS leaching potential index	0.15	-0.49
Soil degradation (days) (aerobic)DT_{50} (typical)	50	12
Soil degr.(days) (aerobic) DT_{50} (lab at 20°C	76	49

registrated with the application dose of 1000 ml/da and 1000 g /da, respectively. Generally weigh of 1 da soil is known 250 tones. After necessary calculations, 500 g sandy-loam soil is treated with pesticides. 2D apparatus divided 2 equal part with wooden rod. 100 g of pesticide applicated soil was transferred through right part of system, and 100 g untreated control soil was transferred through left part. Soil moisture was equilibrated to the field capacity (Figure 1). Later on 3 earthworms, weighed approximately 0.75 g, were released mid of the system and the top of the apparatus were taped to prevent escape (Figure 2).

Experiment were carried out 3 replication for each pesticide application dose. With 3 application dose (recommended dose x 1, recommended dose x 2 and control), 9 2D apparatus were used for each pesticide in the experiment. Totaly 18 pieces of 2D glass apparatus were incubated 3 days at 18 ºC.

Figure 1. Placing of pesticide treated soil on the top of 2D (2 Dimensions) glass apparatus and water absorption of soil

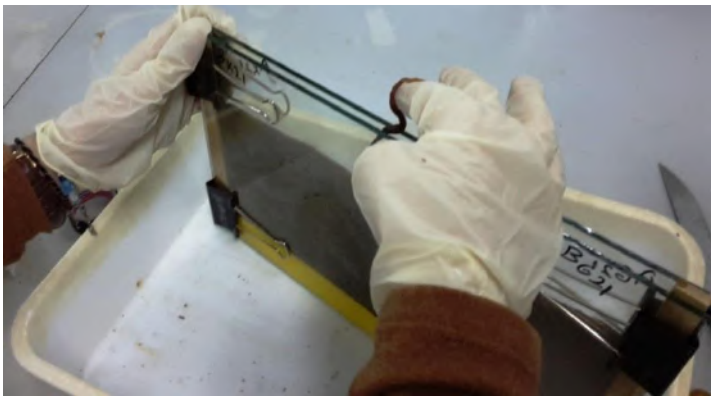


Figure 2. Releasing of earthworm on the 2D (2 Dimensions) glass apparatus.

Results and Discussion

After 3 days incubation, galleries of earthworm were fixed on acetate film with different colour marker (Figure 3). Area of each gallery was measured by using Sketch Up drawing program. Gallery areas in left (treated) and right (control) side were calculated separately. Average area of front and back side was used for calculation.

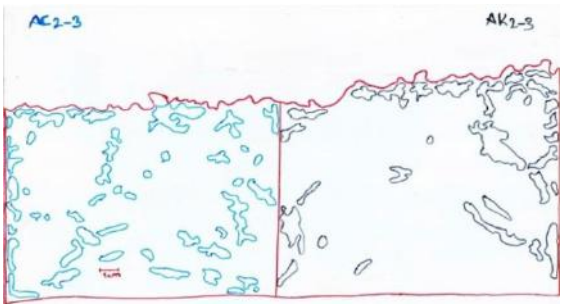
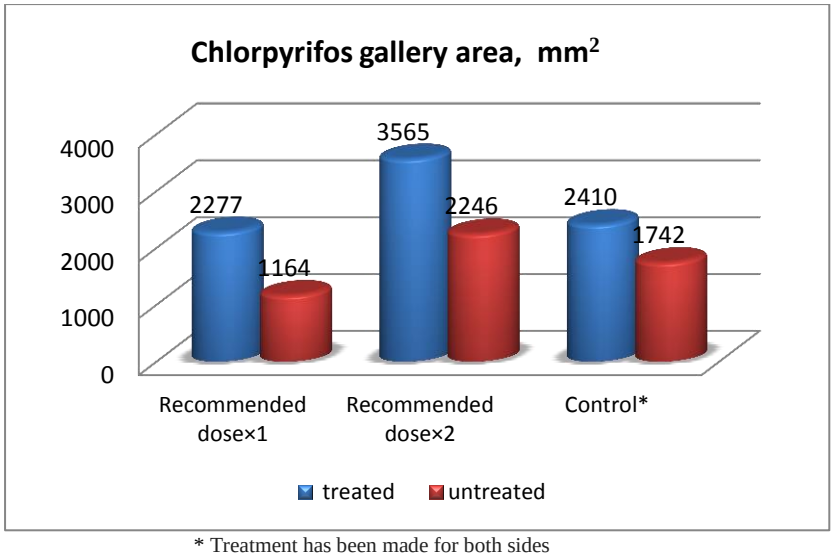


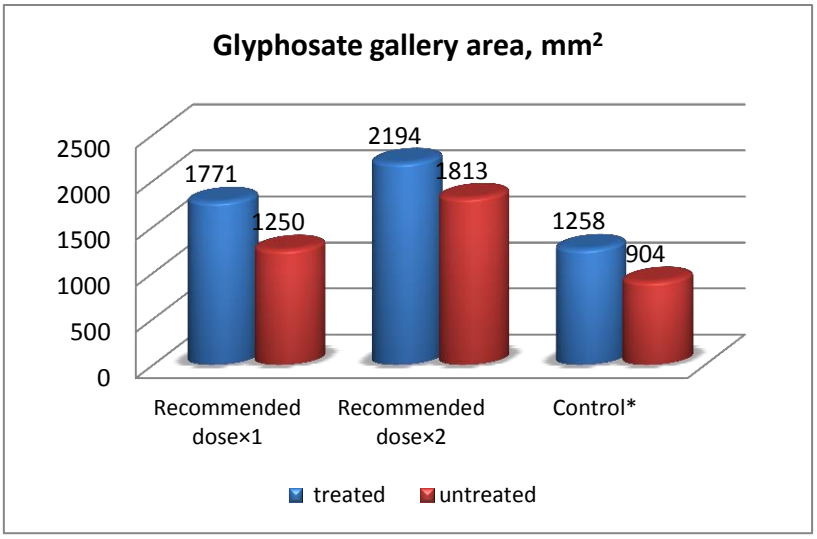
Figure 3. Fixed galleries on the acetate film.

Graphical presentation of gallery area is shown for both chlorypyrifos and glyphosate for recommended dose x1, recommended dose x2 and nontreated in Fig 4, Fig 5 respectively.



* Treatment has been made for both sides

Figure 4. Gallery area of chlorpyrifos experiment



* Treatment has been made for both sides

Figure 5. Gallery area of glyphosate experiment

Statistical assessment was performed with SASV8 variance analysis program. Variance analysis table and calculated data is shown in Table 3. Mean square of replication and treatment, error and CV can also be seen in the table.

Mean gallery arae of chlorpyrifos and glyphosate experiment and statistically significant group, Least Significant Difference (LSD) and F value were given in Table 4. There was no significant differences between recommended dose x1 application and untreated ones, for both active ingredient. At the recommended dose x2 application, there is significant differences at 5% level between treated and untreated ones for chlorpyrifos, where as differences is not significant for glyphosate and control (both side is untreated) experiment. The another interesting finding for both pesticides is that the gallery area of treated (recommended and its twice) experiment is less than untreated control experiment

Table 3. ANOVA data for experiment.

Pesticide dose	Mean square		Error	CV%*
	Replicate	Treatment		
Chlorpyrifos				
Recommended dose x1	63.375	1858153.500	286512.875	31.11125
Recommended dose x2	502515.167	2609641.500	6162.000	2.701098
Control	218080.6667	669002.0417	65740.667	12.34817
Glyphosate				
Recommended dose x1	317217.3750	409770.6667	50895.542	14.92066
Recommended dose x2	13275.2917	216980.1667	130770.5417	18.04652
Control	282706.7917	187797.0417	334726.292	53.49152

*Co-efficient variation

There is a contrast between literature findings and our findings for both pesticides. The main reason of it, 3 days incubation is not enough for this kind of experiment. Booth et al (2000) examined effect of chlorpyrifos and diazinon on *Aporrectodea caliginosa* earthworm and they applied 12 weeks incubation. Capowiez et al. (2006) and Pelosi et al. (2014), applied 1 month incubation for imidacloprid-earthworm experiment. It was stated that high herbicide dose are toxic for soil living organisms (PSU, 2003). The another reason may be the pesticide amount is not enough for affecting earthworms. There is more than one application in the field condition. There is only one application at both dose and its twice in our experiment.

Table 4. LSD and F values of mean areas for chlorpyrifos and glyphosate experiment

Pesticide döşe	Untreated average area, mm^2	Treated average area, mm^2	LSD	p>F
Chlorpyrifos				
Recommended dose ×1	1164.00 a*	2277.00 a	1880.5	0.1258
Recommended dose ×2	2246.67 b	3565.67 a	275.77	0.0024
Control	1742.50 a	2410.30 a	900.76	0.0858
Glyphosate				
Recommended dose x1	1250.7 a	1773.3 a	792.56	0.1050
Recommended dose x2	1813.7 a	2194.0 a	1270.4	0.3266
Control	904.7 a	1258.5 a	2032.5	0.5320

*figures followed by different letters in same line differ significantly at $p< 0.05$. LSD: Least Significant Differences.

Conclusion

Our experiment was performed at controlled conditions in the laboratory. Pesticides is applied more than one in practices. Pesticide amount in the soil is an important factor in earthworms and pesticide relationship experiment.

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ANIMAL HUSBANDRY

Original scientific paper
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IN VITRO EVALUATION OF CLORHEXIDINE BASED DISINFECTANTS USED POST-DIPPING AGAINST MASTITIS PATHOGENES

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Abstract

The selection and use of the most effective disinfectants in the post-milking teats disinfection process has a great importance in the reduction of teat infections, and this is what pushed us towards this undertaken study.

The assessment of the antimicrobial activity of the chlorhexidine based disinfectants, in the post-milking teats treating process was done throughout an “in vitro” method. The disinfectants efficacy was assessed with different active principles to the most common mastitis causes, preliminary defined in some cattle farms of Korca region.

The disinfectants Alpha Blue, Chlorhexidine digluconate 20%, used as solution with 5000 ppm active principles digluconate 20% used as solution 4500 ppm active substance.

The disinfectant effectiveness was defined as a percentage ratio between the destroyed microorganisms and those recovered by control teats. The chlorhexidine based disinfectants tested in our study resulted well and concretely their efficiency of Alpha Blue for four pathogenic microorganisms (Streptococcus agalactie, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa) was: 93.4%; 98.4%;97.6%;96.0% and Deosan: 97.3%;96.1% 95.4%; 94.0%. Chlorhexidine disinfectants have a very good antibacterial effect against mastitis causes. Results obtained through out this study match the nowadays literature data.

Key words: *Mastitis, in vitro, teats, disinfectants.*

Introduction

Mastitis, mastos in Greek, *μαστός*=teat and itis=inflammation, can be defined as an inflammatory state of the mammary gland that is accompanied with pathological changes of her tissues as well as with physical, chemical, cytological and bacteriological milk changes. Bovine mastitis is a mammary gland caused mainly from bacteria and favored by certain environment circumstances. Great economic loss in livestock farms come as a result of the reduction of milk production and changes in its physic-chemical composition as well as the treating cost.

Mastitis is called clinical when clinical signs are present in mammary gland tissues. For sub-clinical mastitis, the only indicator is the high number of somatic cells in milk, without any apparent anomaly in teats issues and milk. It can be said that for each clinical-mastitis in stall there are 10-20 sub-clinical mastitis.

Practically, all bacteria are mastitis causes in case all hygiene conditions favor their development. Main bacteria are classified in: infective Streptococcus agalactiae, Staphylococcus aureus, Corynebacterium bovis and Mycoplasma sp. s, environmental streptococcus and Gram-negative and opportunistic Coagulase negative.

Staphylococcus aureus. It is faced in a half of sub-clinical mastitis and about one third clinical mastitis.

Streptococcus agalactiae is one of the most widespread pathogenic germs. It could be found in a half of clinical mastitis and one third of sub-clinical ones.

Bibliographic facts show that mastitis epidemiology is changing, such as, e.g. Streptococcus uberis actually represents a redeemable clinical mastitis case. It was considered such an environmental microorganism but now shows contagious abilities as well in stalls. While Pseudomonas species are rapidly becoming prevailing mastitis in stalls.
Despite the antibacterial barrier of teat sphincter, the transfer of some microorganisms is inevitable.
Factors that favor mastitis are:
Improper environment hygiene, of: stall, animal, milkmaid, milking apparatus.
The neglect of post-milking teats disinfection.
Use of not effective disinfectants.

Material and Methods

The control and prevention of mastitis, is also the aim of this study. Animal teat health preservation through the sink of post-dipping teats with the most effective disinfectants prevents mastitis. There can be used a lot of disinfectants but the most common ones according to the consulted literature are the iodine and chlorhexidine.
The used disinfectants are: Alpha blue, chlorhexidine digluconate 20%, is used as a solution with 5000 ppm active principles (chlorhexidine based product).
Deosan, contains chlorhexidine digluconate 20%, used as a solution 4500 ppm active substances.
Disinfectants antibacterial activity was tested for the most frequent mastitis causes in some cattle farms in Korca region and concretely:
Streptococcus agalactie, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli.
For each of the above microorganisms we got clean microbial culture.
36 samples (teats) were taken in the slaughterhouse slaughtered for various reasons, with healthy mammary glands and were preserved at a temperature of -20 ° C.
The antibacterial activity was evaluated through laboratory tests using teats experimentally contaminated with pathogenic microorganisms described above.
Microorganisms were obtained from a former cultivation and they were transferred in triptosio agar in 200 ml PBW (peptone broth water) and stored at: 37 ° C for 48 hours “Streptococcus agalactie” and 24 hours “other organisms”. After incubation, suspension was obtained through centrifugation 4000 rpm (rotations / minute); it was cleaned 2 times with Peptone-based water and then diluted (10 based dilution) till a concentration of 10^8 CFU / ml broth was reached. After the dilution, it was made the cultivation 0.1 ml in blood agar ground/ land. In addition, the broth culture obtained was melted in sterile milk dive in which there were merged all the previous melted (in warm water at a temperature of 40^{o}C) teats. Thus, all the teats became infected.
For each microorganism there were used 3 controlled dovetail/ teats and three experimental dovetail/ teats for each disinfectant (3x2). Just the experimental teats (6 for each microorganism) were plunged, 3 in each disinfectant.
After 10 minutes, all the nipples were cleaned with 5 ml broth Bacto- lethen liquid; the cleaning liquid was collected for each disinfectant. It was taken 0.1 ml liquid from the cleaning of the controlled teats for each micro organism and it was made cultivation on blood agar at 37^{o}C for each disinfectant. Likewise the controlled teats, it was made the cultivation of 0.1 ml of the cleaning liquid of experimented teats separately for each microorganism and for each disinfectant. After 24-48 hours, the colonies were counted.
The effectiveness of disinfectants action was determined by the percentage ratio between the microorganisms destroyed in the teats treated with disinfectant and those recuperated in control teats

Results and discussion

Table 1.Testing with Pseudomonas aeruginosa

Disinfectants	Infection dose M	Survived Microorganisms M	Destroyed Microorganisms M	Effectiveness %
Alpha Blue	T. Contr. $1.0x10^8$	$0.04x10^8$	$0.96 x10^8$	96.0
	Exp. T. $1.0x10^8$			
Deosan	T.Contr. $1.0x10^8$	$0.06x10^8$	$0.94x10^8$	94.0
	Exp. T. $1.0x10^8$			

Deosan and Alpha blu disinfectants acted with a high destroying effect against Pseudomonas aeruginosa.
T.Contr.-Teats of Control
T.Exp.- Experimental Teats

Table 2. Testing with Escherichia coli

Disinfectants	Infection Dose M	Survived Micororganisms M	Destroyed Microorganisms M	Effectiveness %
Alpha Blue	T. Contr. $1.3x10^8$	$0.024x10^8$	$1.271x10^8$	97.6
	Exp. T. $1.3x10^8$			
Deosan	T. Contr. $1.3x10^8$	$0.046x10^8$	$1.254x10^8$	95.4
	Exp. T. $1.3x10^8$			

While testing Escherichia coli Deosan and Alpha blu disinfectants had a high effectiveness.
T.Contr.-Teats of Control
T.Exp.- Experimental Teats

Table 3. Testing with Staphylococcus aureus

Disinfectants	Infection Dose M	Survived Microorganisms M	Destroyed Microorganisms M	Effectiveness %
Alpha Blue	T. Contr. $1.29x10^8$	$0.02x10^8$	$1.27 x10^8$	98.4
	Exp. T. $1.29x10^8$			
Deosan	T. Contr. $1.29x10^8$	$0.039x10^8$	$1.24x10^8$	96.1
	Exp. T. $1.29x10^8$			

Deosan and Alpha blu disinfectants were very effective in destroying the microorganisms.
T.Contr.-Teats of Control
T.Exp.- Experimental Teats

Table 4. Testing with Streptococcus agalactie

Disinfectants	Infection Dose M	Survived Microorganisms M	Destroyed Microorganisms M	Effectiveness %
Alpha Blue	T. Contr. $1.1x10^8$	$0.072 x10^8$	$1.028 x10^8$	93.4
	Exp. T. $1.1x10^8$			
Deosan	T.Contr. $1.1x10^8$	$0.028x10^8$	$1.070x10^8$	97.3
	Exp. T. $1.1x10^8$			

Deosan was more effective than Alpha Blue against Streptococcus agalactie.
T.Contr.-Teats of Control
T.Exp.- Experimental Teats

Table 5.The destroying percentage of mastitis causes from disinfectants in test

Disinfectans	**Streptococcus agalactiae**	**Staphylococcus aureus**	**Escherichia coli**	**Pseudomonas aeruginosa**
Alpha Blue	93.4%	98.4%	97.6%	96.0%
Deosan	97.3%	96.1%	95.4%	94.0%

Conclusions

Deosan, containing chlorhexidine digluconate 20%, used as solution 4500 ppm active substance. (17500 ppm), is more efficient in our case. Alpha blue, that contains a large amount of active principles chlorhexidine digluconate 20%, is used as a solution with 5000 ppm active principles (chlorhexidine based product).

Experimented method (in vitro) can define the antimicrobial action of used products in the post-milking teats disinfection. Data obtain from this study match to the nowadays literature data in this field.

Chlorhexidine based products tested possess a high antibacterial activity against the routine agents that cause mastitis in cattle.

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Original scientific paper
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COMPARATIVE ASSESSMENT OF PRODUCTIVE AND ECONOMIC PERFORMANCE IN SEVERAL COMMERCIAL BROILER FARMS IN ALBANIA

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Abstract

The study was conducted at seven commercial intensive broiler farms in Albania, respectively in the area of Fier, Lushnja, Kavaja, Durres, Tirana, Vlora and Kruja, during April – May, 2012. All farms were growing Cobb-500 chicken. Number of chicken at the start of growth was the same for all farms (5000 chicks). At the end of the growth period (age 38 days), performance indicators and technical efficiency of growth were assessed. The lower values of mortality rates and feed conversion ratio (FCR) were obtained in the farm located in Kruja. Vlora farm headed for final body weight (BW), the average daily gain (ADG), higher values of performance index (PI), European Production Efficiency Factors (EPEF) and European Broiler Index (EBI). Higher economic efficiency of Vlora's broiler farm was influenced by performance indicators BW, ADG as a result of the strong correlation (0.84) among them. In all farms, subject of this study, performance indicators proved to be inferior compared to hybrid's standard. In Vlora's farm, the value of BW, ADG and FI (feed intake) were respectively 3.6%, 3.7%, and 4.5% lower than the standard, while the value of FCR was 10% higher.

Keywords: *performance indicators, technical and economic efficiency*

Introduction

Intensive/commercial broiler production is considered an important source of provision of animal origin protein. Broilers provide have a good meat quality and a high rate of productivity (FAO, 1999). Genetic improvement in terms of improving broilers' performance has substantially shortened their finishing time (Gunasekar, 2006). Because of the short growing cycle, broiler production ensures high returns and income (Kleyn, 2002; Grepay, 2009). In the next decade poultry meat will be the cheapest and most favored one, being marketed to customers in cuts. But this requires the finished broilers to be of higher body weights, higher meat yield, and consequently higher value added (Schmidt, 2008). In the past, poultry industry mainly focused on the dynamics of growth and feed conversion. Feed conversion rate (FCR) is considered as one of the most important parameters to assess the capacity of hybrids, or their nutrition program (Leeson, 2005). Researchers are studying options to improve feed conversion and growth indicators, in order to minimize the cost of production (Ghazi AM et al., 2012). Due to the substantial changes occurred in this area, to better evaluate the performance of broilers it is real important to take into consideration both biological performance and economic parameters. In order to accurately measure the broilers' performance several indices are calculated. Many of them represent only the biological performance of the birds; they can be used to measure the genetic capacity of birds, food quality and technical efficiency of the farm (Samarakoon S.M.R et al., 2012). Although there are many commercial broiler farms in Albania, full details regarding technical and economic indicators of production as well as an analysis of these indicators are not available. Therefore, the ***goal*** of this study is the evaluation of production and economic performance indicators in several commercial broiler farms.

Materials and Methods

The study was conducted in seven commercial broiler farms in Albania (in areas of Fier, Lushnja, Kavaja, Durres, Tirana, Vlora and Kruja), during April – May, 2012. Inclusion in the study of these farms was not random. Farms were selected considering their technology related conditions, growing broilers of the same hybrid (Cobb 500). In each farm for this study's purpose, a unit with the same number of heads - 5,000 birds was targeted. In all farms the chicken were kept in floor. The study was conducted during one growing cycle, from 1 to 38 days. Each farm had its specific conditions in terms of growing conditions and technology's implementation.

Performance parameters investigated during the monitoring for each unit were: final body weight (BW), average daily weight gain (ADG), feed conversion ratio (FCR), the mortality, as well as the economic efficiency of growth, through the calculation of PI (performance index), European Broiler Index (EBI) by Van, I. et al., 2003, and European Production Efficiency Factor (EPEF) by Broiler Management Manual Ross-308, 2009, and Van, I. et al., 2003.

Mortality was recorded daily, while based on the obtained data the percentage of viability was calculated. For analysis of performance indicators such as:

BWG (body weight gain), ADG (g/chicken/day), FCR, viability, PI, EPEF and EBI the following formulas were used:

BWG (grams on period) = BW (g) at the end period - BW (g) in first day;

$$\text{ADG (g/chick/d)} = \frac{\text{BWG}}{\text{days number of growth period}}$$

$$\text{FCR (kg feed/kg gain)} = \frac{\text{Feed intake (kg)}}{\text{BWG(kg)}}$$

Viability (%) = chicks remaining at the end of period (%);

To better judge on the performance of each farm, the index of production (IP) was computed. The Performance Index (PI) (Jahan MS, M. Asaduzzaman and AK Sarkar, 2006) was calculated as follows:

$$\mathbf{IP} = \frac{\text{Live Weight}}{\text{FCR}} \text{x} 100$$

European Production Efficiency Factors (EPEF)

$$(\mathbf{EPEF} = \frac{(\text{Flock Livability x Average Live Weight}) \text{ x } 100}{(\text{Average Kill Age x Feed Conversion})}$$

European Broiler Index (EBI)

$$\text{EBI} = \frac{\text{Livability (\%)x ADG (g/chicken)/day)}}{\text{FCRx10}}$$

EBI values are always lower than EPEF values, because for calculating the ADG, the Body Weight at age at day 1 day is excluded (Van, I. et al., 2003).

The obtained results according to the methodology were statistically processed for average values. To study the linkage between technical and economic indicators several correlations were calculated.

Results and discussion

Growth Indicators:

Throughout the growth period of the chicken, the mortality in each farm was monitored. At the end of growth (38 days) the number of chicks in each farm was recorded as in the following table:

Table 1: Mortality in each farm

	Fier	Lushnja	Kavaja	Durres	Tirana	Kruja	Vlora
Housed chicks in the Day 1	5000	5000	5000	5000	5000	5000	5000
Mortality (%)	9,12	3,96	5,62	6,48	4,96	1.68	3.92
Finished broilers	4544	4802	4719	4676	4752	4916	4804

Table1, clearly shows that the lowest mortality was demonstrated in Kruja's farm,followed by that of Vlora, Lushnja, Tirana, Kavaja, Durres and Fieri.

Analysis of performance indicators (i.e. BW, ADG, FCR and Mortality) is calculated based on the economic efficiency of broilers'growth.

Table 2: Broilers' growth performance indicators according to the respective farm (unit)

	Fier	Lushnja	Kavaja	Durres	Tirana	Kruja	Vlora	Standard
BW kg/chick	1.840	2.060	1.930	1.840	1.990	1.800	2.200	2282
ADG (g/day)	47.4	53.2	49.7	47.4	51.3	46.3	56.8	59
FI kg/chick	3.434	3.653	3.580	3.432	3.607	3.190	3.420	3.826
FCR	1.96	1.81	1.91	1.93	1.86	1.79	1.90	1.71
PI (%)	93.88	113.81	101.05	95.34	106.99	100.56	115.79	136.16
EPEF	224.52	287.56	250.90	234.76	267.51	260.26	292.77	
EBI	219.78	282.28	245.59	229.68	262.13	254.31	287.23	

The highest Finished Live Weight and Gain were received in ***Vlora's*** farm, followed by Lushnja's, Tirana's, Kavaja's, Fieri's, Durres' and Kruja. Although the broilers grown in Kruja's farm showed a satisfactory vitality, we can't say the same about the growth dynamics. Although Vlora's farm had 24.2% higher mortality than the Kruja's one, its broilers had an average weight of 400g (or 18.2%) higher.

The data obtained from this monitoring related to the production indicators were compared with the standard Cobb 500 (Broiler performance and Nutrition supplement; Cobb 500, 2008) for the same age (38 days).

It turns out that the closest to the standard body weight and gain were achieved at Vlora's farm. However, the values obtained in this farm were respectively 3.6% and 3.7% lower than the standard.

During the cycle, less feed per bird (FI = feed intake) is consumed in Kruja's farm, followed by Vlora's, Durresi's, Fier's, Kavaja's and Lushnja's ones. The same can be said for FCR, which shows a lower value in Kruja's farm, followed by Lushnja's, Tirana's, Vlora's, etc. FCR is strongly related to vitality (-0.88) which is clearly demonstrated in Kruja'sfarm (Table 3).

In all farms is consumed less feed per bird compared to the hybrid's standard. Closest to the standard is Lushnja's farm, with 173g (or 4.5%) less. In all farms, chicken were fed ad-libidum. Lower feed consumption in all monitored farms, should be connected with its quality (nutritional values, of the feed formula).

Kruja's farm broilers have used the feed per unit of weight more effectively, but in comparison with the standard have a higher FCR (4.5%). Given that in all farms, chicken have not received sufficient amount of feed, their growth dynamics and hence the final weight

has not been satisfactory. This has led to the acquisition of higher values of FCR than the standard's one. So feed is not used effectively.
As a result, the recorded values received by this study in all farms were inferior compared to the hybrid's standard.
Out of the data on growth performance: BW, ADG, FI, FCR and vitality, the values for the economic efficiency of growth, were obtained, through the calculation of PI, EBI (Van, I. et al., 2003) and EPEF (Van, I. et al., 2003 and Broiler Management Manual ROSS-308, 2009; Marcu, A., et al, 2013).
The highest IP was in Vlora's farm, followed by Tirana's, Lushnja's, etc. As lower the FCR's value is and the greater the body weight, the greater will the value of PI. Because in the calculation of this index, the relations between the IP and the Body Weight are the strongest (0.92), the farms that have the highest indicator will lead. However, at the farm of Vlora there is a blizzard of 14.96% in the IP's value compared with hybrid's standard.
In many countries the EPEF is used to measure the growth performance of broilers (Ross Broiler Management Manual 308, 2009; Van, I. et al., 2003). The EPEF value describes the overall production profile (Szollosi L, et al, 2014). This simple index facilitates the comparisons within a farm and among farms and can be used to assess changes in the application of the microclimatic and management parameters (Samarakoon S.M.R et al, 2012). The higher the value of EPEF is, the better is the technical performance of the birds. In our case, Vlora's farm has demonstrated the highest EPEF values, followed by Lushnja's, Tirana's and Kruja's ones. A broilers' flock with acceptable growth parameters and vitality should reach the EPEF values of 200-225 (Ross Broiler Management Manual 308, 2009). According to Reenivasaiah P.V.S, 2008, the optimal EPEF should have a value over 250. In our case, only four farms (Vlora's, Lushnja's, Tirana's and Kruja's) demonstrate to have this value over 250. Since EPEF includes the body weight and the finishing age at slaughter, (as well as the dynamics of growth), but the vitality and FCR as well, this indicator will also reflect any negative or positive effect related to health, environmental stresses or quality of feed. Lower values of EPEF in Kavaja's, Durresi's and Fieri's farms, show the necessity for a more accurate assessment of production parameters, including a study of data concerning the management and the health problems, in order to be able to determine the possible causes of a low output.
Vlora's farm has demonstrated to have the highest value of EBI. EBI is used to predict the efficiency of broiler production. Economic analysis based on EBI is used to predict farm income as well (Reenivasaiah P.V.S, 2008).
The highest value of the two indexes (EPEF and EBI) in the holdings of Vlora, Lushnja, Tirana and Kruja shows that their performance is better than the other farms. In both indices (EPEF and EBI) the highest influence belongs to the respective factors: Body Weight (0.84), ADG (0.84), Vitality (0.71) and finally FCR (-0.60).

Table3: Correlations between performance indicators and technical efficiency of growth

	FCR	Livability	BW	ADG	PI	EPEF	EBI
FCR	1						
Livability	-0,87936	1					
BW	-0,09084	0,257548	1				
ADG	-0,09046	0,254393	0,999962	1			
PI	-0,47436	0,564477	0,919443	0,919456	1		
EPEF	-0,60922	0,714302	0,840247	0,839545	0,980835	1	
EBI	-0,60294	0,706183	0,84526	0,844629	0,983	0,999927	1

Vlora’s farm and the one of Tirana and Lushnja are characterized by better indicator of technical efficiency of growth. This statement is verified and supported by the strong positive correlation (0.98-0.99) between these three indices (EPEF, PI and EBI). The higher is the productivity efficiency factor (EPEF) and European Broiler Index (EBI), the more rentable and the more profit will be farms.

Conclusion

Based on the results obtained out of the seven farms involved in this study, the following might be concluded:

Although Kruja’s farm is differentiated for best indicator of vitality and FCR has not managed to meet the desired performance because of insufficient feed consumption;

Vlora’s broilers demonstrated to have higher body weight and higher gain compared to other farms, but these parameters were lower compared to the standard (3.6 and 3.7% respectively).

Lower feed consumption/bird on all farms during the growth period can probably be linked to its quality, which led to inferior body weights and higher FCR, compared to the hybrid’s standard;

Vlora’s farm has shown a better technical performance (PI), EPEF and EBI (respectively 115.79, 292.77 and 287.23), compared to other farms subject of this study, resulting to be the most profitable farm.

For farms with poor indicators of technical efficiency of growth, one should make a fair assessment of production indicators related to growing/keeping conditions, feeding and health management of the flock.

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PLANT EXTRACT EFFECT OF *YUCCA SCHIDIGERA* ON OOCYST SHEDDING IN BROILERS

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Abstract

In Algeria, coccidiosis constitutes one of the main constraints which hinder the development of the poultry production and is responsible for major losses to the poultry industry, reducing performance and increasing production costs, especially increase of mortality and misuse of antibiotics. These molecules have fatal effects on the animal and public health, Thus, alternatives were introduced, in particular the extracts of plants. The present study, conducted in October 2013, was carried out in five hundred broiler chicks (Hubbard F15 strain). The chicks were monitored from birth to the 52nd day. These animals with homogeneous weight of 93 g, and mixed sexes, came from the same hatchery and underwent the same environmental conditions. Animals of the first lot, identified as "Control lot" received a food exempt from any additive but water added of antibiotics. The animals of the second lot, identified as "Experimental lot" received a water exempt from any additive and the same food added with the *Yucca Schidigera* extract. The objective of this study was the evaluation of the oocystale excretion by Mac Master's method to estimate the activity of the *Yucca Schidigera* extract. The results highlighted an increase of oocystale excretion until 82250 oocysts.g^{-1} of feces in the control lot and 22950 oocysts.g^{-1} of feces in the experimental lot. The average levels of oocysts were significantly lower during three weeks from J14 to J35 in the experimental lot, then remained comparable for the rest of the experiment period (J36 to J51). These results showed that this additive considerably reduced the coccidian eggs elimination and proved its efficiency in the coccidiosis control.

Keywords: *Yucca Schidigera, coccidiosis, broiler chicken, antibiotics.*

Introduction

Poultry is a significant economic protein source, especially for developing countries, which justified its rapid development across the globe for thirty years (Sanofi, 1999).

Poultry farming in Algeria has experienced spectacular growth and expansion through the various development plans of the Ministry of Agriculture. The increase in production is due to a control of livestock management, improved nutritional optimization of diets (Langhout, 1998) and the control of the health status animals. Coccidiosis is the major problem in poultry production with a significant economic impact, estimated at 2 billion$ including mortality (6 to 10%), the cost of prevention and treatment. The etiologic agents is an intestinal parasitic protozoa, coccidia of Eimeria which 7 species infect chicken (Yvore 1992). The control of this disease in livestock is essential to the success of the poultry industry. In Algeria, the treatment of coccidiosis is based on the use of chemical anticoccidials (antibiotics) in the food and drinking water. However, because of the prohibition of these antibiotics, the concern to maintain a satisfactory level of production requires the search for non therapeutic solutions that replace antibiotics. Alternatives to antibiotics should be both effective on the zootechnical, health and economic parameters. Additives proposed, are organic acids, essential oils, probiotics and prebiotics (Dorman and Deans, 2000). The objective of this

study is to evaluate the effect of supplementation based on *Yucca Schidigera* plant extract having various biological activities, antioxidant, anti-inflammatory, antimicrobial and antiparasitic can intervene in maintaining the health of the animal.

Material and Methods

The experiment was conducted for a period from November 2013 to January 2014. Five hundred day-old chicks of Gallus gallus domesticus belonging to the broiler strain Hubbard F15, were put in place in November 2013 and followed from the 1st to 52nd day. Animals were raised in a traditional style building located in the wilaya of Tipaza, compartmentalized so as to provide two living areas of 180 m². Animal's weight were 48 g of mixed sex from the same hatchery in the same environmental conditions. Food formulation considers the three breeding phases (starting, growing and finishing). The water served to the animals came from a regularly treated drink. Animals of the first batch, identified as "control lot" were receiving free food, any additive but water with antibiotics, the most frequently antibiotics administered on Algerian territory, from 1 to 14th day (day 1 to day 14) Animals of the second batch, identified as "experimental lot" were receiving free food, any additive but a drink of water with a anticoccidial based *Yucca schidigera* (Yuquina XO) produced by the NORFEED company, Europe. A vaccination protocol chicks was established and followed by the veterinary livestock. Animals in both lots were vaccinated against Newcastle disease KINGDOM L CEVA® at D6 and booster with L NEW CEVA® to J19 and also against infectious bursal disease IBD L CEVA® at15th day.

Experimental Protocol

We realized a comparative study between two lots: o The control group (TEM) with 250 chicks, receiving a food and water containing antibiotics.

o The experimental group (EXP) 250 chicks fed with the same food that the control but water with the *Yucca Schidigera* liquid. Harvesting of droppings was conducted in the barn to make a Fecal examination by highlighting the presence of oocysts in the feces of broilers in order to diagnose coccidiosis (Mayot, 2005).

Feces several subjects were collected in sterile jars from the 14th day (because the oocyst excretion begins on the 8th day) until the 51st day. The operation is done every day in the morning, collecting fresh droppings spread around the breeding of two lots in order to have a representative sample. These droppings were put in pots identified on the date of collection, the letters "TEM" for the control batch and the "EXP" for the experimental group. Droppings samples were transported immediately to the laboratory for parasitological analysis.

Counting oocysts of Eimeria

In the laboratory, we proceeded to parasitological diagnostic by the quantitative method. This method is based on counting oocysts in feces by Mac Master method. This method consists in counting the number of parasitic elements contained in 0.30 ml of a diluted fecal suspension at 1 / 15. This method relies on the use of Mac Master blade and has a flottation principe. Then, an amount of about 1 kg of salt is poured into a pan containing 1.5 liters of water. If necessary, add salt to saturation. We have carried out the preparation of the suspension of faeces.

The Mac Master blade is filled with the suspension of feces and placed under a microscope and retained about 10 min. The observation is made with a microscope (X10).

OPG of the two lots was calculated each day of the experiment (J14 to J51).

Results and discussion

Counting oocysts

The results of the count oocysts from 14th to 51st day show that the maximum values for each blade of the control group were 1645 and 658 oocysts, and from 21st to 31st they are 459, 198 and 237 oocysts J23, J27 and J41 respectively for the experimental group respectively.

b. Counting OPG

Results for both lots exhibit maximum values for the control group of 82250 and 32900 oocystes.g-1 faeces at 21st and 31st day respectively; these values were 35900, 9900 and 11850 oocystes.g-1 faeces at 23th , 27th and 41st respectively for the experimental group.

The results of the number of OPG of the two lots are illustrated in the following figure:

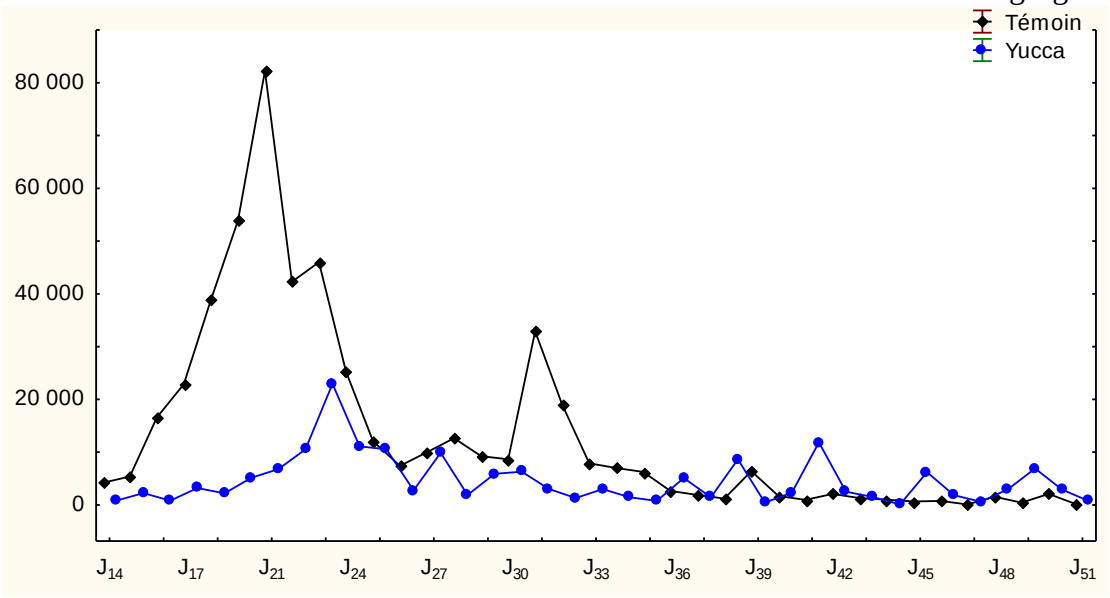

Figure 1: Evolution of oocyst shedding subjects of each lot.

Results show for the control group a progressive and significant increase in oocyst shedding from 14th (4200 OPG) and reach a first peak at 21st (82250 OPG) then decrease at 26th (7400 OPG) shedding then stabilized between 27th and 30th (9250 OPG) to move up to a second pic at 31st (32900 OPG). Then we have a lower concentration at day 33th (7900 OPG) and stabilize again from day 34th (7000 OPG).

For the experimental group, we have a stabilization of oocyst shedding between 14th (800 OPG) and 18th (2200 OPG) followed by a slow increase and reach a first peak at 23th day (22950 OPG) and then decease at 26th day (2750 OPG). The oocyst shedding present a second peak at 27th (9900 OPG), and decrease than at 29th (5900 OPG) followed by stabilization at day 30 (6300 OPG).

Comparison (2-2) the number of ovocytes.g-1 (mean ± SE) between control and experimental samples (paired samples) shows significantly differences for the first 3 weeks of measures (Table 1), corresponding to day 14-21, 22-28 and 29-J35.

Then levels ovocytes.g-1 was comparable for both samples at day36 to 51.

Table 1: Average weekly levels ovocytes.g-1of two lots

		Control	Experimental	p
D_{14}-D_{21}	1ère week	32014±10748	3029±845	0.03
D_{22}-D_{28}	2ème week	22236±6059	9957±2617	0.03
D_{29}-D_{35}	3ème week	12993±3692	3107±839	0.04
D_{36}-D_{42}	4ème week	2414±698	4621±1587	0.33
D_{43}-D_{49}	5ème week	829±188	2879±1013	0.10
D_{50}-D_{51}		1150±950	1800±1100	0.14

This difference in the number of oocystes.g-1 feces (OPG) between the two lots (TEM, EXP) is explained by the rise of OPG in the control group after a heavy infestation of coccidia. We registered a decrease in oocyst shedding after the administration of a chemical anticoccidial (Toltrazuril, Baycox®). This medication was made at the onset of clinical signs at D21 and D31.

Moreover, the number of OPG is weaker in the lot EXP with no symptoms compared to TEM lot. This seems to be explained by the incorporation of the extract of *Yucca schidigera* in their diet from day one.

However, Dahmani and Djaouchi (2013) made with the same additive and Trigonella Graecum in broilers report two relatively small peaks substantially similar for both lots between 18th and 20th day. These authors concluded that the use of the plant *Yucca Schidigera* and *Trigonella Graecum* extracted in broiler feed effectively protects animals against coccidiosis.

Various publications have reported efficacy studies of herbal products on the prevention of coccidiosis in vivo. Allen et al. (1997) demonstrated that the extract of wormwood leaves used as food additives would have a protective effect against intestinal lesions produced by the parasite. Recently, Naidoo et al. (2008) showed that the extract of this plant reduces significantly the production of oocysts to poultry, and restores their weight gain. Moreover, Saini et al. (2003a, b) and Giannenas et al. (2003) reported that the essential oil of oregano reduce the expression of coccidiosis in chickens. However, the mechanism responsible for these beneficial effects remains unexplained.

Mpoame et al. (2003) studied the effect of aqueous extracts of papaya seeds in older chickens (37 days), inoculated with a suspension having a oocyst who received 10 days later aqueous extracts based treatment papaya seeds administered for 3 days with doses of 0, 10, 20 and 40 gl-1; they revealed that the OPG were similar in all items and showed a reduction of OPG between samples before and after treatment.

The number of OPG 0 gl-1 was significantly higher ($p < 0.05$) for both post-treatment samples compared to the batch that received 40 gl-1, but comparable to lot wich receive 10 gl- 1. Conversely, the study Kouakou et al. (2010) using the aqueous extract of Thonningias anguinea revealed a presence of oocysts in the feces of all infected batches. This presence has been constant in the untreated batch infected with a higher excretion 1.2x105 OPG during the study. By cons, in both treated groups, we observed a reduction of oocysts excreted with OPG (contaminated and treated with conventional anticoccidial Super hipracox-p).

Conclusion

This study evaluate the effect of the *Yucca schidigera* plant extract on oocyst shedding in broiler chicken, we concluded that supplementation of this extract in the experimental group feed from the first days of life the chicks reduces significantly the elimination of coccidia eggs.

We also noted the absence of pathognomonic signs of coccidiosis, with this anticoccidial (*Yucca Schidigera*) introduced preventatively in broiler breeding. Whereas for the control group, we concluded that the use of anticoccidial early did not prevent the onset of the disease and its evolution.

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NATURAL PASTURE EFFECT ON THE FATTY ACID COMPOSITION OF THE CAMEL'S HUMP

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Abstract

The aim of this study was to determine the value of fatty acids in the hump of camel. The fatty acid composition of the hump of 43 camels (*Camelus dromedarius*) with ages ranging from 1 to 13 years was determined by gas chromatography. The saturated fatty acids in the hump represented 64.4% of the total fatty acids, while the monounsaturated and polyunsaturated fats accounted for 33.1 and 2.5%, respectively. The main saturated fatty acids in the camel hump, namely the palmitic and stearic acid represented 49,6% and 38.8 % of the SFA (31.5% and 25.5% of the total fatty acids). The unsaturated fatty acids were mainly represented by the oleic acid, totaling 78.1% of the monounsaturated ones (25.9% of the amount of total fatty acids), the linoleic acid which accounted for 88.5% of the ω6 (1.17% of the total fatty acids) and the linolenic acid accounting for 63.9% of the ω3 (0.42% out of the total fatty acids).

The saturated fatty acid/unsaturated acids ratio have equalled 0.039 and that of ω6/ω3 equal 2.81.The results showed that the levels of fatty acids, saturated fatty acids and monounsaturated fatty acids were significantly higher in females, while the content of ω6, ω3, Linolinic Conjugate Acid (CLA), polyunsaturated (PUFA), as well as the PUFA/saturated fatty acid (SFA) and ω6/ω3 ratios were comparable in relation to both sexes.When reported to the breeds, the contents of ω6 and PUFA were higher in the Sahraoui, same as the ω6/ω3 ratio. The percentages of SFA, however, were higher in the Tergui, while the percentages of monounsaturated fatty acid (MFA) and ω6 were higher in the Sahraoui.

Keywords: *Hump, camel, fat acid, breed.*

Introduction

Camels store energy as fat deposits accumulated in various parts of the body, mainly hump and abdomen (Kadim et al., 2002). But their originality at this level resides in the preponderance of the hump. This one contains most of the subcutaneous fat, while the internal fat is mainly located around the kidneys (15% of the fat storage) and inside the mesentery (5%) (Kamili et al., 2006). As a whole, a dromedary weighing 750 kg contains more than 150 kg of body lipids. Changes in body fat are very little known in what concerns the dromedary. On average, the weight of lipids in the hump is estimated between 10 and 20 kg, but with a large variability associated to the physical activity and the nutritional and physiological status - age and sex- of the animal (Wilson, 1984). For example, the size of the hump increases during the rainy season, when the animal restores its fat deposits (Chilliard, 1989).

The lipid metabolism underlying fat store in dromedary is known (Mirgani, 1977). Its indicators are the same as in other species: determination of cholesterol, triglycerides, free fatty acids at serum levels, but also the fatty acids composition of the hump (Chilliard, 1989; Kadim et al., 2002).

Most of the studies performed to date on fatty acid composition of the hump in dromedary concerned animals raised in intense farms. In Algeria, dromedary is generally kept on natural pasture. Their diet includes foliage and desert vegetation. The dromedary is fed on *Acacia sp.*,

and *Artiplex sp.* and fatty acid profile of their fat must reflect the metabolism associated to poor vegetation from arid zone.
The aim of this study was thus to assess the fatty acid profile of hump fat from dromedary raised on naturel pastures in Algeria.

Material and Methods

Animals and sampling:

This study was performed on 43 animals slaughtered at the Ouargla abattoir (800 km South-Eastern from Algiers-Algeria) in 2011. Animals were represented by 7 females and 36 males aged 1 to 13 years old, belonging to two breeds, i.e., Sahraoui and Tergui.
Directly after slaughter, samples of 50 g fat were obtained from the center of each hump, carried out in ice to the laboratory and stored at -20°C in sealed plastic bags until analysis.
The fatty acids were analyzed based on gas chromatography with electronic pressure control Hewlett Packard type (HP 6890 series), equipped with a capillary column HP-5 (30 m x 0.25 mm) displaying a film thickness of 0.25 µm, an FID detector set at 260°C and fed with a H_2/Air mixture and a split/splitless injector set at 275°C. Injection by split method (split ratio: 1/50). The gas used was nitrogen with a flow rate of 1.7 $ml.min^{-1}$. The column temperature was set to 50 to 25°C at $4°C.min^{-1}$. The unit was controlled by a computer system "HP ChemStation" type, managing the operation of the device and enabling monitoring of the progress of the chromatographic analysis.

Statistical analysis:

The analysis was performed on Statistica 10.0, (Statsoft Inc., Tulsa, USA) and IBM SPSS 20 (IBM Corp).
Fatty acids (SFA, MFA, PUFA) were expressed as % of hump fatty acids (FA). The normality of the values distribution and the homogeneity of variances were evaluated by the Kolmogorov-Smirnov and Levene tests, respectively.

Results and discussion

Proportions of fatty acids:

Weight proportions of individual fatty acids in the hump of the 43 camels are shown in table I.

Table 1: Fatty acids composition (% FA) of hump fat, in algerian dromedaries.

SFA		**MFA**		**PUFA**			
				ω 6		**ω3**	
C14:0	4.90±0.21	C16:1cis9	2.33±0.18	C18:2n-6	1.17±0.07	C18:3n-3	0.42±0.05
C15:0	0.83±0.04	C17:1cis10	0.48±0.02	C20:2n-6	0.032±0.004	C20:5n-3	0.035±0.003
C16:0	31.5±0.7	C18:1trans11	2.63±0.26	C20:3n-6	0.025±0.005	C22:5n-3	0.19±0.02
C17:0	1.34±0.05	C18:1cis9	25.9±0.5	C20:4n-6	0.056±0.003	C22:6n-3	0.015±0.001
C18:0	25.5±1.0	C18:1cis11	1.74±0.06	C22:4n-6	0.043±0.003		
C20:0	0.28±0.02	C20:1cis11	0.009±0.001				
Total SFA	64.4±0.7	Total MFA	33.1±0.6	Total ω6	1.33±0.08	Total ω3	0.66±0.06

SFA represented for more than 60% FA hump. Palmitic acid was the most important of them with close to 31.5%, followed by stearic acid with about 25.5%. The two FA represented more than half of the fat hump (57%) and C14:0 represented a low value of 5% of total FA.
As concerns MFA, oleic acid, predominated with 25.9%. Far behind, was found the C16:1(9) with 2.33%. Two intermediate chain of fatty acid, C18:1 trans 11 and C18:1cis 11 showed closed levels and accounted for about 2.63 and 1.74% respectively.

When regarding PUFA, linoleic acid was predominant with 1.17% (88.5% of the total ω6), linolenic acid with 0.42% (63.9% of the total ω3). The cis-9, trans–11 CLA averaged 0.50%. In much smaller quantities followed C22:5n-3 (0.19%) and C20:4n-6 (0.056%).
Other fatty acids were found in very small quantities. However, PUFA/SFA was 0.039±0.003 and ω6/ω3 was 2.81±0.33.
According to an unpublished study reported by Faye et al. (2012), the dromedary hump stores on average 44% of the fatty reserves of the animal. It is the most representative organ in terms of importance of fatty reserves, before the perirenal and perivisceral regions. The fatty tissue is also deposited in different parts of the carcass like shoulder, sternum, flank, ribs, thigh and neck, subcutaneous and inter- or intramuscular tissues. In fatty animals, the tissue accumulates equally in the ano-genital area (Ollier, 1995; Faye et al. 2002).
The dromedary hump represents almost 13% of the carcass weight (Yousif and Babiker, 1989) and its tissue could contain 84% fat (Abu Tarboush and Dawood, 1993). In that species, half of the sirloin cut may be fat since hump is currently part of this cut (Yousif & Babiker, 1989). Its importance on a human dietary point of view could thus not be neglected.
Our results showed that the fat found in the dromedary hump was characterized by a high content, at about two third, in saturated fatty acids, the monounsaturated acids practically representing one third and the PUFA a level as low as 2%. More than half of the PUFA were from ω6 family, a bit more than a quarter was represented by the ω3 family, and the fifth was represented by cis-9,trans-11 CLA.
When regard to SFA in the hump prevailed palmitic (31.5%), oleic (25.9%), stearic (25.5%) and in a lower amount, myristic acids (4.9%). The same observation, in similar orders of importance, was reported by Mirgani, (1977) and Kadim et al. (2002), Mirgani (1977) having observed a slightly higher proportion in miristic acid (12%). Rawdah et al (1994), as for them, reported that the main fatty acids present in the fat stored in the dromedary hump were palmitic acid (34.4%), followed by oleic (28.2%), myristic (10.3%) and stearic acids (10.0%).
Comparing the saturated fatty acids present in the dromedary hump with those acids present in the milk shows very similar values. The findings of Gorban et Izzeldin (2001) showed that the camel milk is also rich in saturated fatty acids (66, 1%) and monounsaturated fatty acids (30.5%). Accordingly, the dominating saturated fatty acids are palmitic acid (34. 9%), myristic acid (14. 5%) and stearic acid (9. 7%).
It is remarkable to note that these four FA generally stem from a lipogenetic metabolism (Maier et al., 2006) or, in ruminants, from saturation of dietary fatty acids in the pre-stomaches (Kouba et Mourot, 2011). Owing to the fact that the diet from dromedary maintained on pasture is, as a rule, very poor in fat, it can be concluded that the very large proportion of the fat found in the hump derive from the lipogenesis in the animal.
In man, the saturated fatty acids consumed in excess increase plasma cholesterol, LDL and HDL levels (Mensink et al. 2003). However, not all saturated fatty acids bear the same cardio-vascular risk: the medium chain fatty acids such as myristic or palmitic acid have more deleterious effects than the long chain fatty acids such as stearic acid (Dubois et al., 2012). In our results, medium and long-chain FA accounted for similar amounts, and the mono-unsaturated fatty acids practically represented one-third of the total fatty acids (33.1%), with emphasis oleic acid, representing the very large part of the mono-unsaturated acids and also the second most frequently encountered FA after palmitic acid, i.e., more than a quarter of the total FA. Similar values have been reported by Mirgani (1977), Emmanuel and Nahapetian (1980) and Kadim et al. (2002). In lower quantities, but consistent when put in relation to the mono-unsaturated acids, trans-vaccenic, palmitoleic and vaccenic acids totalized close to 7% of total FA.

Mono-unsaturated fat acids are considered as beneficial for health owing to the fact it decreases the total cholesterol and LDL and, in the same time, increases the HDL blood levels (Hu et al., 1997).

Short chain PUFA levels were quite low in the present study. Besides similar features as ours for SFA and MFA, Emmanuel and Nahapetian (1980), as well as Kadim et al. (2002) also reported low levels of C18:2 w6 and C18:3 w3 in hump fat.

Polyunsaturated fat are protective against cardiac arrhythmias. A study of post-menopauseal women with a relatively low fat intake showed that polyunsaturated fat exert a protection against the progression of coronary atherosclerosis, by contrast to monounsaturated fat (Mozaffarian et al., 2004). However, it was known that polyunsaturated fats do are sensitive to lipid peroxidation. Vitamin E has been shown to be protective against this phenomenon (Leibovitz et al., 1990), as well as selenium, whose levels in meat from dromedary are rather high (Sahraoui et al., 2013). The unsaturated/saturated fatty acids ratio was 0.039 and that of ω6/ω3 2.81. These ratios could be judged small because the current recommendations for a healty diet are in favor of ratios close to 1 and 5, respectively (Renaud and de Lorgeril 1989; ANC, 2011).

Conclusion

This study showed that hump of two breeds of dromedary (Sahraoui and Tergui) raised on natural pasture in Algeria is especially rich in saturated fatty acids, followed by mono unsaturated ones, with modest levels of polyunsaturated onesThe fat of Sahraoui breed appeared to be richer in unsaturated fatty acids. Hump fat may be considered as a significant source of unsaturated fatty acids for nomadic populations.

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EFFECTS OF FOOD SUPPLEMENTS FOR YUCCA SCHIDIGERA ON LIPID BALANCE OF BROILER

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Abstract

The purpose of this study was to investigate the effects of *Yucca schidigera* on animal and lipid parameters in broilers. For this, animals were divided into two equal groups of 950 chicks. Y*ucca schidigera* supplementation was incorporated into a standard diet of experimental group (0.5 kg/tonne) for a period of 52 days.

Zootechnical parameters (weight gain, feed conversion and mortality) and lipid parameters were determined at the end of each breeding phase (day 28, 42, 52) (total cholesterol, HDL-cholesterol-cholesterol LDL, triglycerides).

χ^2 test showed that mortality rates in 2 batches (control and experimental) were comparable for the 3 periods (p=0.26, p=0.11, p = 0.20). The results of weight gain obtained at the end of breeding showed a difference in weight between the animals of the ”control” and the “experimental" groups (2270 vs 2240 g), but without significant statistical difference.

In parallel, net decreases in triglycerides and LDL levels were observed in all supplemented animals. These results showed that dietary supplementation with *Yucca schidigera* interfered with the energy and fat metabolism of the animals.

Keywords: Yucca schidigera chicken production parameters, lipid profile.

Introduction

Poultry farming in Algeria has expansed through the various development plans (1).

Antibiotics as growth factors are among the most commonly used additives to improve feed efficiency and growth animals rate and consequently increase the productivity of farms. However, they have encouraged the emergence of antibiotic residues in the food chain and human health (antimicrobial resistance) (2). However, following the ban on the use of antibiotics as growth promoters from January 2006 by the European Union and in order to maintain a satisfactory level of production, more additional ingredients to the diet improves disease resistance and participate in their properties and bioactive molecules to improve health of animals, have been undertaken recently as an alternative of antibiotics, including enzymes, organic acids, extracts of natural plants, probiotics and prebiotics (3).

In Algeria, to fight against coccidiosis, we use the chemical coccidiostats in feed and drinking water.

The aim of this study was to evaluate the effect of dietary supplementation of anticoccidial containing the natural extract "*Yucca schidigera*" in our broiler farms.

Material and Methods

The aim of this study was to evaluate the impact of food supplementation *Yucca schidigera* extract, on growth performance and blood lipid parameters.

Our study was conducted during the period of February to April 2014, in a traditional farm, located in Tipaza (north of Algeria).

Zootechnical parameters:

1.1. Animals:

We used two thousand seven hundred (2700) day-old chicks of Gallus gallus domesticus, coob strain, mixed sexes, a homogeneous weight (93 g) from the same hatchery. These animals were weighed and divided into two (2) batches. Animals were put in place 10 February 2014 for a period of 52 days, in a traditional building, under the same conditions of environments.

1.2. Food:

The food used was produced especially for our experiment on the basis of a formulation into account the three breeding phases (starting, growing and finishing). Drinking water distributed to the animals came from a regularly treated well.

1.3. Preventive treatment:

Subjects in both lots were vaccinated against Newcastle disease KINGDOM L CEVA® to D7 and a booster with L NEW CEVA® j17 and j45 for and against Gumboro disease IBD L CEVA® at J9 and J20.

This study aims to compare the lipid profiles of two experimental treatments:

The first batch of animals (300), identified as "control group" received the food free of additives but water with antibiotics, the most frequently administered treatments on Algerian territory.

The second batch of animals (2400), identified as "experimental group" received a drinking water free from any additive and the same feed supplemented with *Yucca schidigera* extract in an amount of 0.5 g/kg (this is an anti-coccidian form of a powder "Yuquina" produced by the company NORFEED Europe).

From these two lots 23 subjects were sacrificed, the blood test was done at the age of 28^{th}, 42^{nd}, 52^{nd} days to determine the parameters of the lipid profile.

Biochemical study was to evaluate the effect of *Yucca schidigera* extract on some biochemical parameters of chick blood (total cholesterol, triglycerides, HDL and LDL).

Statistical analysis

Comparison tests of weights between batches "Experimental" and "Control" has been made based on the calculation of RC critical report.

The mortality rates were compared using χ^2 tests.

Otherwise, comparisons of total cholesterol, triglycerides, HDL and LDL were established between experimental and control groups for the three periods of experimentation with Student or Mann-Whitney tests.

The results are given as mean ± SE and differences were considered significant at $p<0.05$.

The analysis was based on SAS or Statistica 10, Statsoft Inc., USA.

Results and discussion

I. Zootechnical parameters:

1. The weight of chicks:

The average weight values (g) of chickens supplemented or not by *Yucca schidigera* extract were given in Table 1.

Table 1: Evolution of the weight of chicks two batches (g).

Lots		**D_{28}**	**D42**	**D_{52}**
Experimental (n = 1400)	Weight (g)	581±7,2	1575±8,7	2510±7,1
	Feed conversion	2.99	1,42	0.65
	Mortality rate (%)	3.25	**4.42**	**67,92**
Control (n = 300)	Weight (g)	862±13,8	1986±10,6	2775±6,9
	Feed conversion	1,72	1,85	1.90
	Mortality rate (%)	**1,66**	3	**49**
	p (Weight)	**<0.001**	**<0.001**	**<0.001**

Chickens from the control group had a higher weight than the experimental group during the three breeding phases with high significant differences ($p<0.001$).
Feed conversion ratios of supplemented group with *Yucca schidigera* extract is lower compared to the control group during the growth phase until age 52nd.

Indeed, the use of anticoccidial based on natural plant *Yucca schidigera* and probiotics in avian husbandry has been the subject of numerous studies where weight gain has improved as reported (Kucukkurt *a*nd Dundar, 2013).
Mortality rate:
Animal mortality observed in the first three days is mainly due to transportation and handling stress during the chicks installation. Therefore, we will not take into consideration, and we have registered mortalities between 4th and 52nd day.
The mortality rates at D_{28} were comparable between “Exp” and “Contr” group ($\chi^2=2.23$, $p=0.14>0.05$)
The mortality rates at D_{42} were comparable between “Exp” and “Contr” group ($\chi^2=1.26$, $p=0.26>0.05$)
There was a highly significant difference between the mortality rates at D_{52} between “Exp” and “Contr” group ($\chi^2=38.70$, $p<0.001$)
According to Villate (5) norms of mortality rates are below 5%, which shows a mastery of breeding practice.
Jang et al. (6) indicated that the weight, average daily gain, the amount of food ingested, and feed efficiency were not significantly improved due to the addition of plant extract

Biochemical parameters:
The samples of chickens supplemented in *Yucca schidigera* extract, showed a decrease in cholesterol levels (table 2) (the difference was not significant $p=0.25$).
At the age of 42nd day, the total cholesterol content in both batches was similar (1.23 and 1.24).
We can see that the voluntary cessation of supplementation *yucca schidigera* extract cause of increased cholesterol level, this might be explained by the positive effect of this extract (the difference was not significant $p=0.14$).
Triglycerides levels were reduced on the 42nd day (0.47 and 1.03) and also the 52nd day between supplemented and control groups (the difference was not significant $p=0.14$ and 0.34).
HDL: it was noted decreased HDL levels in day 42 in the batch supplemented but not significant $p=0.77$).

LDL: in this study, we observed lower levels of LDL cholesterol in supplemented lot. At 52nd day LDL-cholesterol level is high in experimental group compared to the control (1.43 and 0.54) which corresponds to the voluntary cessation of supplementation *Yucca schidigera* extract.

Thus, Zhao et al (7) saponins from Yucca schidigera decrease cholesterol, triglyceride levels, and LDL levels were significantly reduced, in contrast to HDL witch is increased.
However, Kaya et al (8) reported the same observation using *Yucca schidigera* extract, these authors noted a decrease in the cholesterol levels in chickens

Moreover, Kucukkurt and Dundar (4) have shown that supplementation of *Yucca schidigera* induced HDL concentration increase and a decrease of cholesterol rate, triglycerides and LDL lipoprotein.

Table 2: Biochemical parameters.

	D_{28}			D_{42}			D_{52}		
Parameters	**Control**	**Experimental**	**p**	**Control**	**Experimental**	**p**	**Control**	**Experimental**	**p**
Ch T ($g.l^{-1}$)	1.30±0.23	0.94±0.14	**0.25**	1.24±0.29	1.23±0.32	**0.98**	1.40±0.44	2.88±0.66	**0.14**
Trig ($g.l^{-1}$)	0.48±0.07	0.46±0.08	**0.83**	1.03±0.33	0.47±0.02	**0.17**	0.58±0.24	0.32±0.07	**0.34**
HDL ($g.l^{-1}$)	0.82±0.12	0.78±0.12	**0.85**	0.85±0.16	0.79±0.11	**0.77**	0.75±0.15	1.40±0.36	**0.18**
LDL ($g.l^{-1}$)	0.38±0.15	0.06±0.02	**0.11**	0.19±0.08	0.35±0.22	**0.53**	0.54±0.28	1.43±0.32	**0.11**

Ch T: cholesterol total, Trig: triglycerides.

Conclusion

Growth factors antibiotics used in animal feed are responsible of antimicrobial resistance in animals and human.
To preserve the quality of chicken meat (drug residues), which not require a waiting period and therefore consumer health. *Yucca schidigeraa* natural extract was studied on a broiler in order to better determine its effectiveness.

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EFFECTS OF REMOVING VITAMINS AND TRACE MINERALS FROM CHICKEN FINISHER DIETS ON ASH CONTENT IN THE BREAST MEAT

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Abstract

A chicken diet must consist of the following: energy, proteins, i.e. amino acids, fat acids, vitamins and minerals. Having in mind that broiler nutrition costs are high (55-70%), there are permanent searches of the opportunities to reduce them. In support of that, there are many researches aiming to remove some nutritive matters for a certain period of time from a diet with no bad impact on production parameters. The experiment was set up with 1400 chicken, divided into 7 groups, which vitamin additives or vitamin + trace minerals were excluded from the day 30 to 42. These additives were excluded 12; 8 and 4 days before the end of the fattening process. The assumption was that the body of chickens accumulate a sufficient amount of vitamins and trace elements and their exclusion from diet at the end of fattening period had no negative impact on the production characteristics. Great quantitative of water soluble vitamins cannot be generally accumulated, but broiler chicken will continue to use them from natural nutritive, after exclusion of premixes. The monitored production parameter was ash content in the chicken breast meat. The results show that exclusion of both, vitamin and mineral additives led to significantly lower ash content in chicken breast meat. The removing only vitamins did not have negative effects to the research parameter. Duration of the exclusion these substances (12, 8 and 4 days) had no effect on ash content in the breast meat of chicken.

Keywords: *removal, mineral, vitamin, ash content, breast meat*

Introduction

Vitamins and trace elements in the chicken body have important functions and therefore the feed must contain them in the sufficient concentrations. An excess of these substances in the body can be partly deposited in certain organs and tissues or removed from the body. The high concentration of vitamins and trace elements in the body can cause poisoning or depression, and the lack of these substances leads to the production decline and the appearance of characteristic deficiency symptoms. Many researches aiming to remove some nutritive matters for a certain period of time from a diet with no bad impact on production parameters.

Studies on pigs have shown that the exclusion of trace elements in the final stages of fattening had no adverse consequences on the production performance (Kim et al., 1997; Mavromichalis et al., 1999; Shelton et al., 2004). The studies that are linked to short-term exclusion of vitamins and/or trace elements from broiler chicken diet have shown the possibility of excluding these nutrients without harmful production consequences through some period of time (Summers, 1997; Ruitz and Harms, 1990).

Baker (1997) concluded that the concentration of trace elements in edible parts of the chicken meat will not be reduced as a result of a seven-day exclusion of trace elements from a chicken diet. The same author noted that the elimination of Fe from broiler diet for a period of 7 days did not reduce the concentration of this trace element in the muscle (Baker, 1989). Removing of a vitamin premix from broiler diet at 29 days did not have negative effect on the immune system (Alahyari-Shahrab et al., 2011). The research of Charuta et al. (2013) showed that in a group of male animals, reducing of relative bone mass from 1.03 to 0.79%, during post

hatching development can lead to deformities and fractures of the tibia, which could have negative effects on broiler production.
The removing of zinc from the chicken diet in the last 7 days led to a reduction of its concentration in the bones and intestines, but not in the muscle (Emmert and Baker, 1995).
Zapata et al. (1998) performed the studies on broilers in order to determine the effect of the exclusion of vitamins and trace elements from the chicken diet during the period 21-42 on the content of trace elements in meat. The conclusion was that the exclusion of vitamins and trace elements from the final chicken diet had no adverse effect on the mineral content in chicken meat. However, the calcium content was lower with the exclusion of these supplements.
Chicken breast meat has less fat and connective tissue in relation to other parts of chicken carcass, and because of that it is better digested. In addition, this type of meat is a good source of mineral substances, primarily richer source of K, Mg and P (Poltowicz and Doktor, *2013).*
The aim of this study was to investigate the effect of removing of vitamin-mineral or vitamin supplements from diet on ash content in the breast meat, only in the last stages of chicken fattening period. We should bear in mind the importance of chicken breast meat as a source of vitamins and minerals in human nutrition. On the other hand, the highest increase of chicken weight gain happens in the last period of fattening. Therefore, the possibility of exclusion of vitamin and mineral supplements without negative effects on the mineral content in meat could lead to significant financial savings.

Materials and methods

The experiment was set up in the building for chicken fattening, on the private "Vujat" farm, Srbac municipality. Total of 1400 one day old chicken, Cobb 500 hybrid, were divided into seven groups, each of 200. Chicken were fed with two mixtures whose nutritive matters were compatible with recommendations of the producers of line hybrids. The first mixture (PI) was identical for all chicken and it was used for the first 15 days. Afterwards, up until 30 day of fattening the second mixture was used (PII), which also was the same for all groups. From day 30 the same mixture as from 15-30 days were used, but vitamins and trace minerals or both were removed. After the day 30, the following mixtures were used: 1) full premix in concentration of 1% with vitamins and trace minerals according to the needs of this category (control group); 2) mixture with 0,5% premix, with only trace minerals (NV); 3) mixture with no premix, with no vitamins and trace minerals (NVM). Control group of chicken was fed with a full mixture, with vitamins and trace minerals up until day 42. NV mixture was given to the groups: II (30^{th} -42^{nd} day), III (34^{th} – 42^{nd} day), IV (38^{th} – 42^{nd} day). NVM mixture was used with following groups: V (30^{th} -42^{nd} day), VI (34^{th} –42^{nd} day), VII (38^{th} –42^{nd} day). On the day 30, each chicken was marked by a leg ring, groups were harmonised as per mass and sex. All health measures have been implemented as per veterinary program.
After completed fattening, 10 chickens was sacrificed from each group (5 male + 5 female) and ash content in breast meat was analysed.
The results were processed by Analysis of variance method using Statistical Programme STATISTICA 10, STATSOFT. Significance of differences between average values of individual groups was determined by F and t test. Because full diet was given to only one group for entire period (control), and NV and NVM mixtures were given in three different periods: 4; 8 and 12 days, it was not possible to make processing by using only one model of variance, it was done separately with using two Analysis of variance models, 3 X 2 (three mixtures, two sexes) and 2 X 3 X 2 (diet with no vitamins; diet with no vitamins + trace minerals; three removals in different times 12, 8 and 4 days; two sexes).

Results and discussion

Ash content was determined in in breast meat of chicken, bearing in mind different mixtures and sex, and it is shown in table 1.

Table 1. Ash content in breast meat of chicken (%), fed with different mixtures of concentrates, monitored depending on sex

Type of diet (F_A)	Sex (F_B)		Feed effect
	Male	Female	
Complete diet (control)	1.18	1.18	*1.18*
No vitamins (NV)	1.29	1.27	*1.28*
No vitamins and trace minerals (NVM)	1.10	1.15	*1.12*
Sex effect	*1.19*	*1.20*	
Analysis of variance			
F_A	F_B	F_{AB}	
25.47**	0.226^{NS}	0.918^{NS}	
t-test significance of differences between the different mixtures of concentrates			
Complete diet 1.18 % (X_1)	t^{1-2}	4.52**	
No vitamins (NV)1.28 % (X_2)	t^{1-3}	2.71*	
No vitamins and trace minerals (NVM) 1.12 % (X_3)	t^{2-3}	7.23**	

By looking data in table 1, it can be seen that statistically there is no a significant influence of sex to tested parameter. However, there is statistical significant impact of type of feed. Chicken that were fed with no vitamins-NV mixture had the greatest ash content in breast meat, 1,28%; chicken fed with complete mixture had ash content of 1,18%, while the lowest content was with chicken with no vitamins and trace elements-NVM, 1,12%. We can conclude that feed with no vitamins and trace minerals influenced the most depressive on the ash content in breast meat.
By monitoring the same parameter, only from the point of length of removal of vitamin-minerals, type of mixtures and sex has been shown in the table 2.

Table 2. Ash content in breast meat of chicken (%) fed with concentrates with no vitamin-minerals from 12; 8 and 4 days

Length of removal (F_B)	Sex (F_C)	Type of feed (F_A)		Effect of length of removal
		No vitamins (NV)	No vitamins and trace minerals (NVM)	
12 days	Male	1.21	1.14	*1.18*
	Female	1.17	1.22	
8 days	Male	1.26	1.10	*1.21*
	Female	1.34	1.13	
4 days	Male	1.40	1.08	*1.23*
	Female	1.32	1.11	
Effect of feed		*1.28*	*1.12*	
Effect of Sex		Male	Female	
		1.20	*1.21*	

Analysis of variance

F_A	F_B	F_C	F_{AB}	F_{AC}	F_{BC}	F_{ABC}
*29.33***	0.744^{NS}	0.191^{NS}	7.233**	1.104^{NS}	0.688^{NS}	1.114^{NS}

Interaction effects

	Type of mixture X length of removal of vitamin-minerals		
	12 days	8 days	4 days
NV mixture	1.19	1.30	1.36
NVM mixture	1.18	1.12	1.09

The above table clearly shows that the ash content in the breast meat of broiler was not depended on the length of the removal of vitamin and mineral supplements, but only on the type of mixture.

Moreover, significant interaction effect was determined by the type of feed and length of removal of vitamin-mineral additives.

These results are not fully consistent with the results of *Zapata et al. (1998),* who concluded that it is possible to exclude minerals without negative effects on meat quality. Also, this is not entirely consistent with the Baker (1997) who concluded that the concentration of trace elements in edible parts of the chicken meat will not be reduced as a result of a seven-day exclusion of trace elements from broiler diet. In addition, Emmert and Baker, (1995) claimed that the removing of zinc from the chicken diet in the last 7 days led to a reduction of its concentration in the bones and intestines, but not in the muscle. It can be said that the exclusion of these substances from the chicken diet in the last phase of fattening had no harmful production consequences, as well as in the researches of Summers *(1997);* Ruitz and Harms, (1990); Shelton et al., (2004). However, it should bear in mind that this type of meat is a good source of mineral substances, primarily richer source of K, Mg and P for human nutrition (Poltowicz and Doktor, 2013*).*

The results of this study show that the exclusion of both together, vitamins and minerals leads to a statistically significant reduction of minerals in the chicken white meat.

Adding mineral substances without vitamins gave even better results on the content of ash in the breast meat. Thus, the mineral content was better than in the group that received the vitamins and trace elements together.

Conclusion

Chicken breast meat has less fat and connective tissue in relation to other parts of chicken carcass, and because of that it is better digested. In addition, this type of meat is a good source of mineral substances, primarily richer source of K, Mg and P.

The highest increase of chicken weight gain happens in the last period of fattening. Therefore, the possibility of exclusion of vitamin and mineral supplements without negative effects on the mineral content in meat could lead to significant financial savings.

The results of this study show that the exclusion of both together, vitamins and minerals leads to a statistically significant reduction of minerals in the chicken white meat. The length of the removal of these substances (12, 8 and 4 days) had no effect on ash content in the breast meat of chicken. Adding mineral substances without vitamins gave even better results on the content of ash in the breast meat. Thus, the mineral content was better than in the group that received the vitamins and trace elements together.

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POSSIBILITY TO USE THREE ACACIA PASTURES

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Abstract

This study evaluated the possibility of using three acacia pasture, which contributes to an increased intake of nectar per hive. Based on field research and visits to 18 beekeepers at three site during the year 2012/2013, obtained results of the daily intake of nectar per hive, which are dependent on the use of one, two or three acacia pasture. The first location related to Bosnian mountainous area (Kotor Varos, Knezevo, Celinac municipalites), second to lowland areas (Srbac, Prnjavor, Banja Luka) and third to the river valleys (Mrkonjic Grad, Sipovo, Ribnik). Daily intake on pasture can be up to 15 kg of nectar, and the total yield from one pasture 70 kg per hive nectar. Acacia honey is of excellent quality, with very pleasant smell and taste. It is one of the most prized honeys. It is almost colorless, crystal clear, with a barely perceptible greenish hue. For a long time does not crystallize, sometimes several years.
Keywords*:* *acacia, beekeepers, bee pasture, nectar.*

Introduction

Honeybee cultivated because of honey, pollen, royal jelly, wax, bee venom and propolis (Đorđević et al. 2004). For production of these products the bee is necessary proteins, carbohydrates, fats, vitamins, minerals and water (Jiang-Guo, 1997). Meanwhile, in the last time, one of the reasons bee mortality is the lack of quality bee food (Mirjanić et al., 2005). Acacia is very extended species with; American origin which entered in 1601 in Europe entered (Maceljski et al., 2001). It blooms profusely, usually from early May until the end of June, 40-45 days after the first movement buds. Start of flowering depends on climatic conditions, altitude, variety, terrain, the degree of insulation areas under the acacia, soil composition, etc. At the time of flowering acacia significantly affect previous climatic conditions, so that the beginning of flowering in the same area year after year can vary up to 15 days. Due to these facts in one season can, with the same bee colonies, to use two, even three acacia pasture. In Hungary, which is a leading country by area under plantations of acacia, long years of work was done to create new varieties of black locust, whose basic characteristics are reflected in better yields nectar, somewhat later onset of flowering and greater resistance to winterkill (Klašnja et al., 2011). Acacia earliest starts to blossom at the lowest altitudes, in the valleys of large rivers. As the altitude increases the land, as a rule acacia on these grounds starts later and later to blossom. The optimum altitude for moving hives to another acacia pasture is 200-300 meters above sea level. Third acacia pasture should be used on hilly terrain, at 500-600 meters above sea level. At one location flowering acacia or nectar secretion usually lasts up to 14 days. Nectar secretion is most intense from the third to the tenth day of flowering. In the lower regions, with fertile soil, acacia blooms earlier and secretes more nectar, but in the mountain. In this case can be, in great part, in the course of a season, the same bee colonies to take advantage of the three locust pasture. The flowers of the acacia strongest secrete nectar on a daily temperature of 20 to 25°C, with optimum humidity and soil when the weather without wind, with the morning dew and temperatures above 16°C. The aim of this work is related to the possibility of three acacia pastures which affects on the increased daily nectar by hive, for the period 2012-2013 years.

Materials and Methods

The study was based on available literature data and an applied field research. Based on field research and visits to18 apiaries during the 2012/2013 at three sites, authors obtained results of the daily intake of nectar per hive, which are dependent on the use of one, two or three acacia pasture. The first location is comprised of six apiaries in mountainous areas (Kotor Varos, Knezevo, and Celinac), the other six apiaries in lowland areas (Srbac, Prnjavor, Banja Luka) and the third six apiaries in river valleys (Mrkonjic Grad, Sipovo, Ribnik).

Results and discussion

On the basis of the research on three sites: mountainous area (Kotor Varos, Knezevo, Celinac), lowland areas (Srbac, Prnjavor, Banja Luka) and in river valleys (Mrkonjic Grad, Sipovo and Pond), data were collected from beekeepers, on the amount of daily intake of nectar with 18 apiaries (locality six apiaries), which has 30 bee colonies, as well as the use of one, two or three acacia pasture during the acacia nectar production.

Tab.1. Daily yield (kg) per hive nectar at the first location and the use of three locust fits

Locality 1	Apiary	Yield (kg)
Kotor Varos	1	15
Kotor Varos	2	15
Knezevo	3	13
Knezevo	4	13
Celinac	5	14
Celinac	6	15

Beekeepers in the first location, mountainous areas used the three locust grazing, moving apiaries to the location where the acacia earliest starts to blossom at the lowest altitudes, in the valleys of large rivers and so use the first acacia pasture. By increasing altitude area, where acacia on these grounds begins later that blooms after 14 days beekeepers move hives to another acacia pasture (200-300 meters above sea level). Third acacia pasture used on hilly terrain (500-600 m above sea level) and so moving around the apiary in stationed. This way of Migration of the apiary, beekeepers exploit the possibility of using three acacia pasture with large living nectar yield per hive.

Tab.2. Daily yield (kg) per hive nectar on another site and the use of two locust fits

Locality 2	Apiary	Yield (kg)
Srbac	1	10
Srbac	2	10
Prnjavor	3	10
Prnjavor	4	9
Banja luka	5	9
Banja luka	6	10

Beekeepers in other localities in lowland areas they used two black locust fits, so that the first apiary moving to a location in the river valleys, where locust earliest starts to copper, and after 14 days apiary returning to the starting point of stationary and thus use two locust fits with smaller daily yield nectar per hive.

Tab. 3. Daily yield (kg) of nectar per hive at the third site and the use of a locust fits

Locality 3	Apiary	Yield (kg)
Mrkonjic Grad	1	5
Mrkonjic Grad	2	5
Sipovo	3	3
Sipovo	4	3
Ribnik	5	3
Ribnik	6	5

Beekeepers third locality in the river valleys moving bee gathering nectar close stationed, and use only a single acacia pasture which leads to a very small living nectar yield per hive. Using three acacia pastures during the year increase the enter of nectar by hive and till five kilograms, although using one or two acacia pastures by hive increases only for two since three kilograms by hive.

Conclusion

Based on the research that refers to the possibility of using three acacia pasture which contributes to an increased intake of nectar per hive for the period 2012-2013.godine can conclude the following:

The results clearly show that during the period of nectar daily intake depended on the possibility of using acacia pasture i.e. there are clear differences between the sites and how to use one, two or three acacia pasture.

The site of mountainous areas showing higher daily intake of nectar per hive from the lowlands and river valleys, due to exploitation of the three locust pasture.

Possibility of three acacia pasture increases daily intake of nectar and up to five kilograms per hive which can be clearly seen from the data collected from beekeepers in the field.

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CHEMICAL COMPOSITION OF EGGS IN DIFFERENT PHASES OF MANUFACTURING OF COMMERCIAL PRODUCTION

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Abstract

The objective of this study was to evaluate chemical composition of eggs (whole egg, shell with membranes, egg white and yolk), a sample of twenty eggs for each stage of the production cycle, and originating from different age of hens during the production cycle (SN20, SN28, SN48 and SN72). Chemical analyses were carried out in two laboratories: the Laboratory of Faculty of Agriculture in Eastern Sarajevo and Food Laboratory Faculty of Technology in Zvornik, University of East Sarajevo. Water content, dry matter, crude protein, fat content, carbohydrates and mineral contents were assessed. Determination of moisture content and dry matter of the samples was made in accordance with current standards by drying in oven kept at a temperature of 105°C. Carbohydrate contents were determined computationally. The content of crude protein was determined by Kjeldahl method. The fat content was analyzed by acid hydrolysis according to the AOAC method, according to the accepted standard. Egg weight slightly increased with increase of hen´s age while the water content and dry matter content were decreased. The crude protein content in whole egg ranged from 12.01% (ALH20) to 12.21% (ALH28), in the yolk from 16.20% (ALH72) to 16.38% (ALH28), egg whites from 10.45% (ALH72) to 10.51% (ALH28). The whole egg fat content was 10.11% (ALH48 and ALH72) and 10.51% (ALH28) in the egg yolk 32.81% (ALH20) and 33.95% (ALH28). Mineral content was the largest in the shell membranes, what is understandable and ranged between 92.36% (ALH20) and 92.63% (ALH48). The chemical composition of eggs and its structural components (shell, egg white and yolk) was similar in all studied stages of the production cycle and was the standard for the chemical composition of eggs produced in commercial production.

Keywords: *table eggs, hens age, chemical composition of eggs, conventional production.*

Introduction

Eggs play an important role in our everyday food. In 2007 total egg production in the world was cca. 62.6 million tons, the European Union produced 9.9 million tons, representing 170 billion eggs (FAO, 2009).

Chicken eggs are one of the most versatile foods. They contain high-quality proteins, carbohydrates, easily digestible fats and minerals, as well as valuable vitamins (Huopalahti, 2007). The egg consists of four different main structures; shell, shell membranes, albumen and yolk. An average egg from domestic laying hens contains 64 % albumen, 27 % yolk and 9 % shell (Rose, 1997). Laying hen eggs are one of the most common and abundant foodstuffs in the human diet and they contain important compounds for human consumption such as lipids, amino acids and vitamins. The yolk is rich in saturated fat, cholesterol and other fatty compounds like lecithin. Small quantities of liposoluble (A, D) and water-soluble (thiamine, riboflavin) vitamins, and minerals like iron, phosphorus, zinc, selenium and sodium are found in eggs too (Belyavin, 1988). Egg quality encompasses several aspects related to the shell (external quality) and to the albumen and yolk (internal quality). The variability in the quality

and nutritional values of eggs has a significant impact on consumers' health; simultaneously, welfare and many other factors can affect egg quality (Sparks, 2006, Zita et al., 2009).
The objective of this study was to evaluate chemical composition of eggs (whole egg, shell with membranes, egg white and yolk), a sample of twenty eggs for each stage of the production cycle, and originating from different ages hens during the production cycle (SN20, SN28, SN48 and SN72).

Material and Method

Chemical analyses were carried out in two laboratories: the Laboratory of Faculty of Agriculture in Eastern Sarajevo and Food Laboratory Faculty of Technology in Zvornik, University of East Sarajevo. The individual is measured by the 20 eggs randomly sampled. Laboratory tests determined the chemical composition of eggs originating from hens of different ages (SN20, SN28, SN48 and SN72). Water content, dry matter, crude protein, fat content, carbohydrates and mineral contents were assessed. Determination of moisture content and dry matter of the samples was made in accordance with current standards by drying in oven kept at a temperature of 105°C (AOAC, 1990). Carbohydrate contents was calculated by using the following formula: 100% – (protein % + fat % + humidity % + ash %). The content of crude protein was determined by Kjeldahl method (mineralization, distillation, titration) described in ISO 59836-1:2006/AC: 2009. The fat content was analyzed by acid hydrolysis according to the AOAC method, according to the accepted standard. During the entire production cycle there were four control individual measurements of eggs (80 eggs in total) and statistical analysis waas donwe on 4 treatments and 20 repetitions (n). The results of analyzes were processed by using standard statistically methods (Hadživuković, 1991).

Results and discussion

Analysis of the chemical composition of eggs and its basic ingredients in the stages of production cycle is presented in Table 1.
Egg weight slightly increased with increase of hens' age, while the water content and dry matter content were decreased (tab. 1). The crude protein content in whole egg ranged from 12.01% (ALH20) to 12.21% (ALH28), in the yolk from 16.20% (ALH72) to 16.38% (ALH28), in albumen from 10.45% (ALH72) to 10.51% (ALH28). The whole egg fat content was 10.11% (ALH48 and ALH72) and 10.51% (ALH28) in the egg yolk 32.81% (ALH20) and 33.95% (ALH28). Mineral content was the largest in the shell membranes, what is understandable, and ranged between 92.36% (ALH20) and 92.63% (ALH48)

Table 1. The chemical composition eggs in the stages of production cycle

Ingredients	SN	Whole egg	Yolk	Albumen	Shell with a membrane
Water					
	SN 20	66.65	48.75	88.01	1.89
	SN 28	65.70	47.55	87.95	1.75
	SN 48	66.61	48.55	87.97	1.81
	SN 72	66.62	48.57	87.99	1.88
Dry matter					
	SN 20	33.35	51.25	11.99	98.11
	SN 28	34.30	52.45	12.05	98.25
	SN 48	33.39	51.45	12.03	98.19
	SN 72	33.38	51.43	12.01	98.12
Crude protein					
	SN 20	12.01	16.29	10.46	5.75
	SN 28	12.21	16.38	10.51	5.78
	SN 48	12.15	16.33	10.48	5.56
	SN 72	12.14	16.20	10.45	5.55
Fat content					
	SN 20	10.15	32.81	-	-
	SN 28	10.51	33.95	-	-
	SN 48	10.11	33.10	-	-
	SN 72	10.11	33.20	-	-
Carbohydrates					
	SN 20	0.91	0.97	0.92	-
	SN 28	1.01	1.02	1.00	-
	SN 48	1.00	1.01	1.00	-
	SN 72	1.01	0.99	0.99	-
Mineral substances					
	SN 20	10.28	1.18	0.61	92.36
	SN 28	10.57	1.10	0.54	92.47
	SN 48	10.13	1.01	0.45	92.63
	SN 72	10.12	1.04	0.57	92.57

Nutritional quality of eggs can be assessed based on compositional quality, which includes crude protein content, ash, fat content, carbohydrates, mineral substances, etc. Commercial eggs are a significant source of fatty acids, cholesterol, and other lipid nutrients in the human diet (Cherian et al., 2002; Hidalgo et al., 2008). The proteins in eggs are easily digested and of good quality, because they contain all the essential amino acids to maintain life and growth. Eggs are a good source of iron, phosphorus, trace elements, vitamins A, E, K, B, D.

When it comes to egg albumen, the biggest constituent is water with a share of 84–89%, and most of the solid matter is represented proteins, 10–11% (Li-Chan and Nakai, 1989). Arias et al. (1993) found that the egg shell contains 3.5% of organic and 95.0% of inorganic matter. Meennicken and Waterloh (1997) in their work found that the dry matter content in egg albumen averaged 12.0%, according to Niewiarowicz (1991), the dry matter content in the egg yolk varied from 50.0% to 54, 5%.

Zanon et al. (2006) examined the chemical composition of egg albumen and egg yolk of two native Italian races of chickens. Albumen moisture content was from 88.27 to 13.29%, the share of albumen was 49.35–52.59% and the proportion of egg yolk was 34.96–32.35%. The

results were compared with the results of two commercial hybrids with white and colored egg shell. The differences were statistically significant ($P<0.05$) in favor of commercial lines. Dziadek et al. (2003) examined the effect of genotype on the chemical composition of table eggs of nine different lines (Lohmann Brown, Shaver 579, AK, ISA White, Mass 445 Mass 443, Astra W-1, W-2 Astra and Astra N), in the period of 63 and 66 weeks of age. The dry matter content in albumen of eggs ranged from 10.98% (Shaver 579) to 13.13% (Astra W–2), and in the yolk from 54.89% (Lohmann Brown) to 59.64% (Messa 443). So, with Lohmann Brown was found uniform, but the biggest moisture of albumen, with a standard deviation of 0.67% and coefficient of variation of 1.22%. The highest values of fat content in the egg yolk was in eggs of genotype Astra N (31.85%) and the lowest value in eggs of genotype AK (29.37%). All differences in chemical composition between the investigated genotypes were significant ($P<0.01$).

Approximate chemical composition of whole egg, according to Li–Chan and Kim (2007) includes 75.84 g of water; 12.58 g of protein; 9.94 g of total fats; 0.77 g of carbohydrates and 0.86 g of mineral substances. Similar results in terms of chemical composition of eggs and its components obtained Jurić et al. (2005), as well as Zanon et al. (2006) who, in addition to eggs produced in the intensive farming system (conventional) determine the chemical composition of eggs produced from indigenous pure breeds in semi–intensive or extensive farming system. Minelli et al. (2007) have reported different values for crude protein, in favour of organically produced eggs, (17.1% vs. 16.7%) compared with those from the conventional system. Many studies have investigated the fatty acid composition of the eggs of commercial layers reared in commercial production system, which include confinement facilities, highly productive hybrid layers kept in limited space (mostly in battery cages) and intensive feeding (Lopez–Bote et al., 1998). To maintain consistently good shell quality throughout the life of the hen, it is necessary to implement a total quality management programme throughout the egg production cycle.

Conclusion

During egg production, special attention was paid to the four main production stages, namely: 20^{th} week – SN20 (start load), 28^{th} week – SN28 ("peak" - maximum), 48^{th} week – SN48 (middle) and 72 week old hens – SN72 (the end of the production cycle). In this period, the adequate number of eggs for analysis the chemical composition of eggs (whole egg, shell with membranes, egg albumen and yolk), with sample of 20 eggs for each stage of the production cycle were taken by random sampling. It can be concluded: chemical composition of the egg and its structural components (shell, egg albumen and yolk) was similar in all tested stages of the production cycle and was the standard for the chemical composition of eggs produced in commercial farming.

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IMPACT OF THIAMINE NUTRITION ON HYPOPHARYNGEAL GLANDS DEVELOPMENT IN IRANIAN HONEY BEES (Apis mellifera meda)

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Abstract

The experiment was carried out under natural condition to assess potential impacts of thiamine nutrition on hypopharyngeal glands (HPGs) development in Iranian honey bees (*Apis mellifera meda*). Twenty honey bee colonies, divided into 5 experimental groups, were used in the study. Control groups were fed sugar syrup (1:1). The experimental colonies were fed in the same manner but with syrup supplemented with different levels of thiamine (100, 200, 300 and 400 ppm). The experiment was conducted for 30 days in the summer. The result showed that the acinal area of HPG showed clear difference under feeding with difference diets. The largest area was recorded when honeybee workers fed the 100 and 200 ppm thiamine at 3 and 6 days of age.

Keywords: *Thiamine, Hypopharyngeal glands, Iranian honey bee*

Introduction

Hypopharyngeal glands (HPGs) consist of a pair of long glands coiled in the sides of the head. HPGs are the main organs responsible of royal jelly secretion. These secretions are fed to the larvae and queens (Crailshem, 1991, 1992). At normal condition they are well developed when bees are nursed and they degenerate when bees become foragers. The size of HPGs is correlated with glandular production and generally increases with age from 6 to 18 days in nurse bees (Hrassnigg and Crailshem, 1998; Deseyn and Billen, 2005). The diameters of HPG are often used to describe the physiological status of worker honeybees. For this reason, gland size and acini diameter have been used as indicators to test the effect of various food sources. The normal course of development of these glands (size of acini) is well known (Maurizio, 1954; Simpson et al., 1968; Moritz and Crailsheim, 1987; Crailsheim and Stolberg, 1989).

Little is known about the vitamin requirements of honey bees, although they are essential for all animals. It appears they are not linked to longevity of the adult bee (Pain, 1956) but are intrinsically linked to brood development (Haydak and Dietz, 1965). The B complex vitamins have been demonstrated to be essential for most insects. Four B complex vitamins, pantothenic acid, thiamine, riboflavin, and pyridoxine, plus vitamin A and K have been linked to development of the hypopharyngeal glands and brood rearing (Somerville, 2005).

Thiamine or vitamin B_1 is a water-soluble vitamin of the B complex. Its phosphate derivatives are involved in many cellular processes. The best-characterized form is thiamine pyrophosphate (TPP), a coenzyme in the catabolism of sugars and amino acids. All living organisms use thiamine, but it is synthesized only in bacteria, fungi, and plants. Animals must obtain it from their diet, and thus, for them, it is an essential nutrient (Du et al., 2011).

The purpose of the present paper is to study the effect of thiamine supplements on development of hypopharyngeal glands in Iranian honey bee (*Apis mellifera meda*) colonies.

Materials and methods

This investigation was carried out at Honey Bee Department of Animal Science Research Institute, Karaj, Iran. Twenty Iranian honey bee (*Apis mellifera meda*) colonies of the same strength and queen of similar age and quality were selected to carry out the study in summer season to test of different diets. Feeding started on July 5, 2012 and continued for 30 days. In this season we prepared five groups of four colonies fed with different levels of thiamine. Feeding treatments were assigned randomly to the colonies in trials. One-year old queens were used in this investigate. Recommended colony management practices were regularly followed in all these colonies.

Thiamine (Sigma Aldrich® thiamine hydrochloride T-4625) was used for feeding in the honey bee colonies. Control colonies received sugar syrup 1:1 (1Lit/day/colony). The experimental colonies were fed in the same manner but syrup supplemented with different levels of thiamine (100,200,300 and 400 ppm).

When the adults emerged, they were marked with atoxic paint and returned to the colony. Hypopharyngeal gland (HPG) was dissected to determine the acini surface at 3, 6, 9, 12 and 15 days old. The HPGs were dissected in sodium chloride solution (0.85%) isotonic to the hemolymph (Al-Ghamdi Ahmad et al., 2011). The glands were not covered by cover glass. For each treatment, we dissected 25 bees, five from each colony. The length and width of ten acini were measured in mm for each worker under stereomicroscope using a micrometer eye piece. The acinal surface was calculated according Maurizio's formula (1954).

$$\text{Acinal surface} = \pi \times \frac{a \times b}{2}$$

Where a = length, b = width, π =3.14.

Data were considered by a one-way analysis of variance for the response variable changes in acinal surface. Differences among means were determined by Duncan's multiple test SAS institute (1991). When the ANOVA indicated a significant effect, post hoc Duncan multiple comparisons test was used to determine individual differences between responses at each time considered, and differences over time for each treatment.

Results and discussion

Means of acinal surface of HPG of the Iranian honey bees after fed different levels of thiamine is presented in Table 1. The acinal surface increased significantly with the age and reached to the maximum at 6-9 days from emergence. These results agreed with those obtained by Hrassnigg and Crailsheim (1998). They reported that peak size is found at around 6 days in bees during the summertime, when workers are known to feed the larvae with royal jelly (RJ). Morphologically, acini size of the HPGs radically changes with age (Deseyn and Billien, 2005). In the present results, it is clear that the greatest acinal area was observed when bees were fed with 100 and 200 ppm thiamine at 3 and 6 days of age. The means area of the acini in the 100 and 200 ppm B_1+SS were 0.0921 and 0.0969 (mm^2) at 3 d, 0.2062 and 0.2147 (mm^2) at 6 d respectively. There was no significant difference between treatments in the acinal area after 6 d. These results confirmed the effect of vitamin B_1 or thiamine on the development of HPGs. The development of HPGs was studied with bees having direct access to brood over the whole period of 15 days. It has often been stated that access to brood is essential for the development of the hypopharyngeal glands (Brouwers, 1982; Moritz and Crailsheim, 1987; Lass and Crailsheim, 1996) but see Crailsheim and Stolberg (1989) who did not find an effect of brood rearing on gland development. Activity and developmental stage are age dependent and can be linked with the behavior of the worker (Wilson, 1971;

Michener, 1974). In conclusion, the gland is fully developed when the worker performs tasks inside the hive for feeding larvae.

Table1. Means of hypopharyngeal gland acinal area (mm^2) under feeding with different levels of thiamine during nursing period of honeybee workers

treatments	3-days old	6-days old	9-days old	12-days old	15-days old
SS* (1:1)	$0.0607\pm0.0075^{bc,B}$	$0.1415\pm0.0151^{bc,AB}$	0.1969 ± 0.0229^{A}	0.1125 ± 0.0394^{B}	0.0992 ± 0.0050^{B}
100ppm B_1+SS	$0.0921\pm0.0008^{a,B}$	$0.2062\pm0.0016^{a,A}$	0.1884 ± 0.0135^{A}	0.1870 ± 0.0248^{A}	0.1112 ± 0.0173^{B}
200ppm B_1+SS	$0.0969\pm0.0008^{a,B}$	$0.2147\pm0.0016^{a,A}$	0.1681 ± 0.0307^{AB}	0.1151 ± 0.0370^{B}	0.1134 ± 0.0229^{B}
300ppm B_1+SS	$0.0519\pm0.0084^{c,C}$	$0.1139\pm0.0169^{c,B}$	0.2067 ± 0.0094^{A}	0.1478 ± 0.0210^{B}	0.1193 ± 0.0250^{B}
400ppm B_1+SS	$0.0808\pm0.0102^{ab,C}$	$0.1777\pm0.0204^{ab,AB}$	0.2041 ± 0.0238^{A}	0.1252 ± 0.0136^{BC}	0.1017 ± 0.0118^{C}

a,b,c Means (± SE) with different superscripts within a column differ significantly at $p < 0.05$.
A,B,C Means (± SE) with different superscripts within a row differ significantly at $p < 0.05$.
*SS: sugar syrup

Conclusion

Our study indicates that thiamine could be affective in development of the hypopharyngeal glands in Iranian honey bees.

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DETERMINATION OF THE SUBSPECIES PURITY OF APIS MELLIFERA MACEDONICA THROUGH MORPHOLOGICAL ANALYSIS OF COLORING OF THE ABDOMINAL RINGS ON THE TERRITORY OF THE REPUBLIC OF MACEDONIA

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Abstract

The Macedonian honey bee (*Apis mellifera macedonica*) is a subspecies of the most famous and also the most widespread type of honey bees in the world, the western honey bee (*Apismellifera L.*). Along with four other subspecies, Macedonian honey bee belongs to the central Mediterranean and Balkan Peninsula group of honey bees. In Republic of Macedonia *Apis mellifera macedonica* is an autochthonous subspecies (race) and is under special mode of protection, which provides continuous control of its subspecies purity which can significantly be compromised if honey bee colonies with queen bees from non autochthonous subspecies are being breed.

The purpose of this research was to determine the purity of *Apis mellifera macedonica* on the territory of Republic of Macedonia through morphological analysis of coloring of the abdominal rings. As a material for our research, we used young worker bees (n= 1440) from 15 apiaries from several regions in the country.

Results have shown that 66.3 % of the analyzed bees were with no colored abdominal rings, 9.5 % were with a single colored ring, 23.5 % with two and 0.7 % with three colored rings. The presence of honey bees with no colored ring indicates that they belong to the autochthonous subspecies *Apis mellifera macedonica*. This percentage of representation of autochthonous honey bee subspecies (greater than 50 %), puts it in the category of none threatened subspecies. But if we analyze the situation in individual apiaries and individual regions, in that case the situation changes significantly and the safety of the autochthonous subspecies is questioned.

Keywords: *honey bees, subspecies, purity, color, abdomen.*

Introduction

The diversity of honey bees (*Apis mellifera* L.), characterized by morphological, ethological and molecular differences in the so far determinate subspecies, is a result of their ability to adapt to the influence of numerous factors and ecological conditions.

Cosmopolitanism is a sufficient proof for the apparent capacity for adaptation of this biologically and economically important subspecies of insects, which during their evolution have gained ability for maximum usage of the existing resources in order to satisfy the rationally developed needs. Different geographic and ecological conditions have led to mutual differentiation of honey bee population and evolution of the subspecies which are best adapted to the given areas. The apparent fall in the number of colonies on global level (Neumann and Carreck, 2010; Van Engelsdorp and Meixner, 2010; Van der Zee *et al.*, 2012; Williams *et al.*, 2012) is a result of the influence of many ecological and anthropological factors. Despite the climate changes and its consequences, one of the most important factors is of course the lack of genetic diversity of the breeding populations (Ellis, 2007) which mainly originate from the limited number of commercial genetic lines for production of queen bees. Additionally, introducing the genotypes of non-autochthonous honey bee population, as a

result of the general communication and transport trends, can lead towards breeding colonies susceptible to various pathogens and stress-factors and obvious reduction of their number both on regional and global level. Therefore, breeding autochthonous honey bee subspecies, due to their adaptability and superior vitality, is the best way to fight against such factors.
In the Republic of Macedonia the autochthonous honey bee subspecies is "Macedonian honey bee" or *Apis mellifera macedonica (*Ruttner, 1988; Stevanovic *et al.,* 2010; Uzunov *et al.,* 2009, 2014) and according to the Law and amending the Law on Livestock Breeding (Official newspaper No 23 February, 14 2013) is under special protection regime. According to the State Statistical Office of Macedonia (2013), the overall population of honey bees bred in the period 2009-2013 ranged between 52 897 and 76 052 honey bee colonies, with a total annual production of 603 to 1 105 tons of honey.
The purpose of this paper work is to determine honey bee subspecies' purity bred in the Republic of Macedonia, since according to some results from morphometric, molecular and ethological researches done by Uzunov *et al.* (2007, 2009, 2013 and 2014), it is possible that there is an influence by other honey bee subspecies in certain regions. This type of influence is an opportunity for hybridization of the autochthonous subspecies, which can lead to a threat as in many northern European countries, where *Apis mellifera* subspecies have perished due to hybridization (Franck *et al.,* 2000; De la Rúa *et al.,* 1998, 2001a, 2001b, 2003; Ivanova *et al.,* 2007).
Studies on this subject, with similar results, previously were made by Kozmus (2011) on populations of Carniolan bees *(Apis mellifera carnica*) on the territory of the Republic of Slovenia.

Material and methods

For our research we used worker honey bees, as part of the 2014 collection from 9 locations of different regions in Republic of Macedonia, from 15 different apiaries and 48 colonies (Table 1). A sample of 30 honey bees has been taken from each colony which has been preserved in ethyl alcohol by the time it's been analyzed.

Table 1. Number of apiaries and number of colonies out of which the material for the analyses has been taken

No	Location	No of colonies
1	Vapila (Ohrid)	3
2	Ezerani (Resen)	3
3	Stenje (Resen)	1
4	Pehchevo	3
5	Josifovo (Valandovo)	2
6	Vinica	1
7	Gostivar I	5
8	Gostivar II	5
9	Gostivar III	5
10	Krstovi (Mavrovo)	5
11	Pelince I (Kumanovo)	3
12	Pelince II (Kumanovo)	3
13	Kumanovo	3
14	Mrshevci (Skopje)	3
15	Drachevo (Skopje)	3

To determine the subspecies' purity of the analyzed honey bees we used the method of morphological analysis on the coloring of abdominal rings.
The color of the abdominal segments (rings) is an indicator for the honey bees' subspecies affiliation. The analysis is done based on the description of the abdominal segments' color given in the Table for the comparison of the main external characters of the principal races of European Bees (http://www.beeworks.com/morphometry). There is no proper comparative table for the Macedonian honey bee with other honey bee subspecies, but since it most resembles to the Carniolan honey bee (*Apis mellifera carnica*) the analysis is done according to the description of this subspecies: the abdominal segments from the dorsum are quite dark with a red and yellow or orange pigment like spots (Figure 1-a, b and c), but at times even a whole colored ring – (Figure 1-d). According to Ruttner, 1988 *Apis mellifera carnica* was allowed to have honey bees with entirely colored ring to maximum 5 % within the colony. The presence of honey bees with two or three colored rings (Figure 1-e and f) shows possible hybridized population, most often with honey bees from the *Apis mellifera ligustica* subspecies, which are characterized by the presence of 1 to 3 yellow colored rings (Rihar, 2003) or hybrids with buckfast bees (Borsuk and Olszewski, 2010).

Figure 1 a) b) c) d) e) f)

The honey bees were analyzes visually, set on a white background, and based on the analysis we divided them into four groups: honey bees with no colored rings, honey bees with a single colored ring, honey bees with two colored rings and honey bees with three colored rings. We calculated the proportional representation of the overall population for each group.

Results and discussion

The morphological analysis of the honey bees from 48 colonies, according to the coloring of their abdominal segments (Table 2) showed that the percentage of honey bees with no colored abdominal rings, which means they belong to the *A. m. macedonica* autochthonous subspecies, is 66,3 %. This percentage of representation of the autochthonous subspecies of the honey bee (greater than 50 %), places it in the category of not endangered subspecies, but if we analyze their state in individual apiaries and regions, then the situation significantly changes and their endangerment is questioned.
The most characteristic example of an apiary is the one in the region of Vinica, where in a single colony all of the analyzed honey bees (100 %) are with colored abdominal rings, 10 % with single colored ring and 90 % with two colored rings.

Table 2. Percentage of honey bees with and without colored abdominal rings

No	Number of analyzed honey bees	% of honey bees with no colored rings	% of honey bees with colored rings			
			One colored ring	Two colored rings	Three colored rings	Total % of honey bees with colored rings
1	90	61,1	15,6	23,3	0	38,9
2	90	97,7	2,3	0	0	2,3
3	30	100,0	0	0	0	0,0
4	90	85,6	8,9	5,5	0	14,4
5	60	10,0	23,4	61,6	5,0	90,0
6	30	0	10,0	90,0	0	100,0
7	150	45,3	9,3	42,0	3,4	54,7
8	150	88,7	6,0	5,3	0	11,3
9	150	90,0	8,0	2,0	0	10,0
10	150	54,0	24,0	21,3	0,7	46,0
11	90	68,8	3,4	26,7	1,1	31,2
12	90	93,3	5,6	1,1	0	6,7
13	90	94,4	4,5	1,1	0	5,6
14	90	72,2	12,2	15,6	0	27,8
15	90	33,3	10,0	55,6	1,1	66,7
Total number of analyzed honey bees	1440					
Average		66,3	9,5	23,5	0,7	33,7

Honey bees with a very high percentage of colored abdominal rings were found in an apiary in the area of Valandovo (Josifovo). In this apiary the proportion of honey bees with no colored rings is 10 % whereas the other 90 % are with colored rings. Although the proportion of honey bees with colored rings is lower than the one in Vinica, yet it is interesting that we found 5 % of honey bees with even three colored rings and high 61,6 % of honey bees with two colored rings.

Except these two apiaries, larger percent of honey bees with coloring in terms of percentage of honey bees with no coloring is found in another two apiaries in the area of Gostivar and Drachevo in Skopje. In the apiary in Gostivar the percentage of honey bees with colored rings is 54,7 %, of which 42 % go to honey bees with two colored rings, 9,3 % honey bees with a single colored ring and 3,4 % honey bees with three colored rings. In the apiary in Drachevo the percentage of honey bees with colored rings is larger and amounts to 66,7 %, but the percentage of honey bees with three colored rings is lower than the one in Gostivar which is 1,1 %.

In the remaining 11 apiaries from which honey bees had been taken for the purpose of morphometric analysis the honey bees with no colored rings prevailed of which 9 apiaries with colored ring honey bees were established with presence of honey bees with a single and two colored rings, and only 2 honey bee colonies were established with presence of honey bees with a single, two and three colored rings.

From the apiaries in which honey bees with no colored abdominal rings prevail we can point out the ones in the area of Resen, where all the analyzed honey bees (100 %) from the apiary in Stenje are with no colored abdominal rings, and the one in Ezerani has 97,7 % of honey bees with no colored abdominal segments.

In the whole analyzed population of honey bees the proportion of honey bees with colored abdominal rings were 33,7 %, of which honey bees with two colored rings prevail (23,5 %), then the ones with a single colored ring (9,5 %) and 0,7 % go to honey bees with three colored rings.

Conclusion

Having in mind the results, the previous findings as well as the current state of the honey bee population in Republic of Macedonia, where the Macedonian autochthonous honey bee (*A. m. macedonica*) maintained the status of unthreatened population, it is necessary to take the following precautions and actions:

Education of beekeepers on the typical morphological characteristics of Macedonian honey bee (*A. m. macedonica*) and creating a practice for queen bees replacement in the colonies in which there is presence of honey bees with yellow colored rings is evident;

Breeding colonies with queen bees produced by the local genotypes of Macedonian autochthonous honey bee (*A. m. macedonica*);

Choosing local genotypes for noncommercial production of next generation queen bees from the Macedonian autochthonous honey bee (*A. m. macedonica*); which are characterizes by superior performances in term of vitality and economically important characteristics;

Providing queen bees from the registered queen bee breeding centers, preferably from their own region, whose breeding programs are focused on improving the genetic basis of local genotypes of the Macedonian autochthonous honey bee (*A. m. macedonica*);

This method is important because is very well applicable, and it can be used by the beekeepers directly on the bee yards. Beekeepers can easily notice the coloring on the abdominal rings, and they can and can respond appropriately on time;

Additional researches need to be done in future referring to the morphometric characteristics of *A. m. macedonica*, with the intention of her protection in the future.

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APPLICATION OF BACTERIOPHAGES IN VETERINARY LABORATORY PRACTICE

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Abstract

The article show using of bacteriophages in veterinary. Bacteriophages are viruses of bacteria. They are unique, nature antibacterial means. Bacteriophages have high specificity to cells owners. This property allows using ones in laboratory diagnostics, and for treatment of animal's infection diseases. The porpoise of our research is allocating bacteriophages to *Salmonella* and *Proteus*, and using them for indication and identification of the same bacteria in food of an animal origin. The objectives of research are poultry forcemeat. Two methods of research were used. First was bacteriological method with application of a standard technique, and second was diagnostics by bacteriophages. Before the start of researches bacteriophages for same bacteria were received, studied their sensitivity, specificity, and the range of lytic action. During the work the control and prototypes samples of forcemeat have been infected by different concentration of *Salmonella* and *Proteus.* After that samples investigated by standard technique and with application of bacteriophages. As a result of the researches sensitive bacteriophages against *Salmonella* and *Proteus* were allocated. Bacteriophages against *Salmonella* showed a wide range of lytic action on different strain of bacteria. Bacteriophages against *Proteus* were specific for *P.* vulgaris. The standard method of research took more time, then a diagnostics with application of bacteriophages. Besides, the bacteriological method of research was demanded a certain experience of work, and statement of diagnostics by bacteriophages was technically simpler for performance and higher sensitivity. Using of bacteriophages in laboratory diagnostics more inexpensive then standard technique of bacteriological method.

Keywords: *bacteriophages, veterinary, Salmonella, Proteus, diagnostics.*

Introduction

Bacteriophages are ultramicroscopic, intracellular obligate parasite, they are viruses of bacteria. For the first time the phenomenon of a bakteriophages was observed by N.F. Gamaley in 1898, later ones was observed by Tuort (1915) and by d'Errel (1917) (Timakov V., Goldfarb D., 1958). Bacteriophages have got heredity, variability, adjustment and properties of viruses. They possess of information necessary for process of their reproduction in the corresponding cells, but have no own mechanism for generation of energy and ribosomes for synthesis of proteins (Katter E., Sulakvelidze A., 2012, Timakov V., Goldfarb D., 1958). Specific interaction of bacteriophages with microorganisms gives chance to use them for identification of bacteria, including pathogenic. Bacteriophages were naturally occur during evolution and can identify owners´ receptors and contacts with cells of the owner only. This fact unlike them from artificially created systems for definition and differentiation of various structures of the bacterial cells. Thus bacteriophages are used in a number of various techniques of specific definition and differentiation of strains of bacteria. The research of objective is comparative analysis of standard bacteriological research and typification by bacteriophages. Bacteriophages to *Salmonella* spp. and *Proteus* spp. were allocated before and then used ones to identification of pathogenic microbes in foodstuffs.

Materials and methods

Materials of research are samples of poultry forcemeat (n = 40). These meat samples have been divided into two groups. The first group were control samples (n=20) and second ones were tests samples (n=20).

Two research methods were used. First is bacteriological standard method and second is identification by bacteriophages.

The bacteriological research directed on detection of bacteria of *Salmonella* spp. was carried out in according to standard (State standard 31468, 2013). The method is based on seeding of 25 g of a product on not selective and selective nutrient medium and the following reseeding on differential gelose substrate. Then it is essential to allocation of the pure cultures having typical for *Salmonella* spp. Morphological and cultural characteristics. Finally it is necessary to identification on biochemical properties and serological reactions.

Indication and identification *Proteus* spp. was carried out in according to standard (State standard № 7702.2.7, 2014). The principle of a method is based on starting raising by peptone-salt solution and then seeding of 0,5 cm^3 ones nutrient agar and 0,2 cm^3 on Ploskirev substrate. After that microorganisms were cultivation at a temperature of 37 ºC during 24-48 hours. In total perform allocation of typical or estimated colonies and identification on typical properties to *Proteus* spp.

Before the experiences bacteriophages against *Salmonella* spp. and *Proteus* spp. were received. For allocation of bacteriophages used horse manure. Placed it in peptone broth in the ratio 1:10 and added 0,2 cm^3 broth culture of *Salmonella* spp. and *Proteus* spp. which were grown to the logarithmic growth stage. Ones incubated at a temperature of 37 ºC during 18 hours, then cleanings of the lysate from impurity, bacteria and their fragments. At a final stage carried out the sterilize filtration by membrane of Millipore Millex-GP firm with a a diameter of time of 0,22 microns. After that sensitivity, specificity, a range of lytic action and a caption of bacteriophages were determination. Then samples of forcemeat were investigated by a classical bacterial method and with application of reaction of increase of phage titre. Estimated labor was input of performance of research; time spent for the analysis; sensitivity of both methods and costs of laboratory testing.

Results and discussion

As a result of the research we allocated sensitive bacteriophages against *Salmonella* spp. and *Proteus* vulgaris (fig. 1).

Fig. 1. Sterility spot on the bacterial lawn

The bacteriophage against *Salmonella* spp. showed a wide range of lytic action. The bacteriophage against *Proteus* spp. was specific concerning *Proteus* vulgaris (tab. 1).

Table 1-Range of lytic action of the allocated bacteriophages on bacterial lawns (according to Adams and Veyd)

Serotype of microorganism	Bacteriophage against *Salmonella* spp.	Bacteriophage against *Proteus* spp.
S.typhimurium	+	-
S.dublin	+	-
S.entritidis	+	-
S.choleraesuis	+	-
S.gallinarum-pullorum	+	-
S.hamburg	+	-
S.virchow	+	-
Proteus vulgaris	-	+
Proteus mirabilis	-	+-
Proteus morgany	-	-

For further work used lysate with the expressed sensitivity. A caption of the received bacteriophages established by Appelman and Gratsia methods. Both bacteriophages showed high titers (tab. 2).

Table 2-Titres of lysates

Serotype of microorganism	Titres	
	by Appelman method	by Gratsia method
S.typhimurium	10^{-9}	$2,1x10^{10}$
S.dublin	10^{-7}	$1,8x10^{11}$
S.entritidis	10^{-6}	$2,8x10^{8}$
S.choleraesuis	10^{-7}	$1,2x10^{8}$
S.gallinarum-pullorum	10^{-9}	$1,9x10^{10}$
S.hamburg	10^{-10}	$1,4x10^{11}$
S.virchow	10^{-7}	$3,5x10^{9}$
Proteus vulgaris	10^{-8}	$1,3x10^{9}$

Then the main objective of research were passed to performance. For this purpose everyone of empirical and control samples of forcemeat weighing 25 g to inoculated to suspension of bacteria in concentration from 10^{1} to 10^{10} cells in one gramme. After homogenized each sample by the laboratory blender and investigated according to State standard and reaction of increase of phage titre. Also obvious advantage using phages are their high sensitivity. (tab. 3).

Table 3-Results of researches control and test forcemeat samples

Concentration of microorganisms, cells/g	Forcemeat samples	*Salmonella spp.*	*Proteus vulgaris*
10^1	Test	-	-
	Control	-	+
10^2	Test	-	-
	Control	-	+
10^3	Test	-	-
	Control	+	+
10^4	Test	-	+
	Control	+	+
10^5	Test	+	+
	Control	+	+
10^6	Test	+	+
	Control	+	+
10^7	Test	+	+
	Control	+	+
10^8	Test	+	+
	Control	+	+
10^9	Test	+	+
	Control	+	+
10^{10}	Test	+	+
	Control	+	+

Conclusion

As a result established that the standard method of research took more time and labor expenses, than bacteriophages used. The time saving when using phages made up 24-48 hours. Thus sensitivity and specificity of bacteriophages deliver of need to carry out morphological and biochemical identification of bacteria. Besides, it should be noted that the bacteriological method of research demands a certain experience of work, and statement of reaction of increase of phage titre is technically simpler for performance. Using of bacteriophages in laboratory diagnostics more inexpensive then standard technique of bacteriological method.

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FATTENING AND MEAT QUALITIES OF BLACK-VARIEGATED BULLS OF DIFFERENT GENOTYPES

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Abstract

Studies on the breeding of crossbred from different genotypes on the Holstein breed on fattening and meat qualities are topical. The aim of this work was to study fattening and meat qualities of bulls of different genotypes. Four groups were formed on the analogue method, 15 animals in each: control group crossbred bulls of genotype $^{3}/_{4}$ H$^{1}/_{4}$ BV; in I, II, III groups - genotypes $^{1}/_{2}$ H $^{1}/_{2}$ BV, $^{5}/_{8}$ H $^{3}/_{8}$ BV and $^{7}/_{8}$H $^{1}/_{8}$BV. Control slaughter of bulls in 18-month age (5 animals from each group) was conducted to study meat qualities. Yield of carcasses and body fat mass were higher than in peers. Weight after slaughter exceeded that of peers by 10.9 kg in the first experimental group. Carcass yield was higher in the control group (by 1.5-1.7%), mainly thanks to higher before-slaughter live weight of bulls. As a result of the research on morphological composition of carcasses and physical and chemical properties of meat of longissimus dorsi no significant differences between the control animals and experimental animals were identified. Thus, the study of fattening and meat qualities of bulls of different genotypes of the Holstein breed showed that breeding animals of the desired type ($^{3}/_{4}$H ¼ BV - control group) does not have a negative impact on meat productivity, and on a number of indicators such as body weight, daily gain, mass of hot and chilled carcasses, weight of flesh and meat of premium, hybrids in the control group outperform their peers in the experienced groups.

Keywords: *bleck-variegated bulls, Holstein breed, genotypes, fattening and meat qualities, slaughter qualities.*

Introduction

To ensure Russia food security it is necessary to reduce the country's dependence on imported food, particularly meat. For this the amount of domestic production of high-quality meat must be increased both for the food processing industry and for the population (Prahov et al., 2004). The main supplier of beef in Russia was and is dairy cattle (Ermilov et al., 2004).

Opportunities to increase beef production from dairy cattle, which produce more than 90% of this type of product, are now investigated. In Russia and CIS countries best domestic and foreign breeding resources of Dutch, Swedish and Holstein origin are widely used for improving black-variegated cattle. As a result, there is a certain genetic structure of herds in the leading breeding livestock farms, which allows working on the creation and breeding of highly productive types of herds, lines, and individual animals. Therefore, breeding of dairy cattle to improve its fattening and meat qualities is of a great economic importance (Prohorenko et al., 1985; Prohorenko et al., 1986; Prozora et al., 1987; Efimenko, 1991; Pashkin, 1998; Prohorenko et al., 2005; Gordeeva, 2005; Czerniawska-Piatrowska et al., 2005; Gordeeva, 2006).

Over the past three decades, a new type of black-variegated cattle was created on the basis of reproductive cross breedind with Holstein breed.

Therefore, studies on the problem of breeding hybrids of different genotypes of Holstein breed for fattening and meat qualities are relevant. The aim of the study was to investigate beef production of Holstein black-variegated bulls of different genotypes.

Materials and methods

Scientific and economic experiment conducted in agrofirm "Zorya", Rovenskaya region (Ukraine), which was one of the basic farms on breeding of a new type of highly productive black-variegated cattle. To create a herd in the farm, mainly Dutch and partly Estonian selections (breeding animals) were used. In recent years domestic production of Holstein breed imported from Canada were widely used.

During the study, all the flock of agrofirm "Zorya" was golshteinized.

Four groups of bulls aged 3 months, 15 animals each, were formed for this scientific and economic experiment by the principle of analogues based on origin, age and body weight. The control group included crossbred bulls of genotype of 3/4 Holstein breed (H) and 1/4 black-variegated breed (BV) which is set by the Ministry of Agriculture of Ukraine as the best for holsteinization. Experimental groups (I, II, III) included the following genotypes correspondingly: $^{1}/_{2}$ H $^{1}/_{2}$ BV, $^{5}/_{8}$ H $^{3}/_{8}$ BV and $^{7}/_{8}$ H $^{1}/_{8}$ BV.

During the period of the experiment (from 3 to 18 months of age) the conditions and feeding were the same for all bulls. Bulls growth monitoring was performed by monthly animal weighing and taking measurements at 6, 12 and 18 months. Diets were composed by growing periods (3-6, 6-12, 12-18 months) with monthly adjustments according to the All-Union Institute of Cattle Breeding norms of feeding (1985). Body weight before slaughter, weight of carcass and visceral fat, slaughter yield, carcass quality, weight of internal organs, and weight of fresh skin were determined according to the control slaughter (five animals in each group) by conventional methods. Morphological composition of animal carcasses was determined by dissection of the left half (three animals for each group) by sausage production technology. Fleshing index was calculated using standard practice. The chemical composition of an average meat sample (ground meat) and longissimus dorsi, and physical and chemical properties (amount of bound water, pH, softness of the meat) of the longissimus dorsi were examined using standard practice.

Results and discussion

Results of the control feeding showed that the level of food consumption did not differ by groups of bulls and was 2801.1-2848.1 food units and 296.1-297.1 kg of digestible protein. 7.04 (control) to 7.32 (I experimental group) food units were spent on 1 kg weight gain during the fattening period.

Analysis of the live weight dynamics in animals of studied genotypes shows some features of each of the experimental groups (Table 1).

Table 1-The dynamics of the live weight of the bulls, kg ($X \pm m_x$)

Age, month	Groups			
	Control	Experimental		
		I	II	III
3	105.4±1.64	102.2±1.60	101.7±2.70	103.9±2.57
6	196.9±2.55	191.5±4.30	189.0±5.05	194.6±4.47
9	309.6±3.48	296.4±5.19	298.4±4.71	305.1±4.55
12	397.0±5.04	382,7±6,36	386.2±6.84	386.6±5.97
15	451.6±5.71	441.9±6.84	439.4±7.33	449.4±5.98
18	490.6±7.07	486.2±7.81	478.7±9.69	485.6±8.24

Bulls of the control group surpassed their peers in the experimental group by 3.7-13.2 kg throughout the experiment. Significant difference in live weight was found between control group and experimental group I at 9 and 12 months, respectively: 13.2 (4.3 %) and 14.3 kg

(3.6 %) at P>0.90-0.95. Animals in experimental group II were inferior in live weight compared to their peers in other groups in all age periods and in 15 and 18 months respectively the difference was: 2.5-12.2 kg (0.6-2.8 %) and 6.9-11.9 kg (1.4-2.5 %).
In the final period of bulls growing and fattening (15-18 months) body weight varied by groups significantly from 478.7 kg to 490.6 kg, and there were no statistically significant differences. It should be noted that by the end of the experiment a slight superiority in bulls live weight (in 0.9-2.4%) in the control group remained.
Average daily gain was higher in the control group and experimental group I (for the period of experiment, 847 g and 845 g, respectively). The maximum average daily gain was obtained in the period of 6-9 months (1179-1265 g). During this period the superiority of animals in the control group over the peers in experimental group I was significant (at P > 0.95).
In general, during the experiment period, the highest absolute increase in body weight was in control bulls (385.5±6.84 kg) and experimental group I (384.3±6.71 kg). Significant difference in the relative growth rate of the animals was found between the control and experimental group I during the periods of 12-15 and 15-18 months (2.3 and 2.0 % respectively, P > 0.99) in favour of animals in experimental group I.
To study the fattening and meat qualities at the age of 18 months, we made a control slaughter of holsteinized bulls (5 animals per group).
Slaughter cattle grading (Table 2) showed that animals in the control group had pre-slaughter live weight 5.6-20.8 kg (1.1-4.2%) higher and fresh carcass weight 10-20 kg (3.3-6.7%) higher than their peers in the experimental groups. Their carcass yield was 1.4-1.8% higher and the weight of visceral fat was 0.1-0.9 kg (0.6-5.6%) higher. Slaughter weight exceeded the similar indicator at peers by value from 10.9 kg (experimental group I) to 20.4 kg (experimental group III), (3.5-6.5% respectively). Slaughter yield in the control group was 1.5-1.7% higher mainly due to a higher pre-slaughter live weight of the bulls.

Table 2-Slaughter qualities of experimental bulls ($X \pm m_x$)

Parameter	Groups			
	Control	Experimental		
		I	II	III
Pre-slaughter weight, kg	496.8±6.5	491.2±6.7	479.6±15.9	476.0±9.3
Fresh carcass weight, kg	299.0±6.7	289.0±6.8	280.0±9.8	279.0±7.1
Carcass yield, %	60.2±0.7	58.8±1.0	58.4±0.4	58.6±0.5
Weight of visceral fat, kg	16.2±0.7	15.3±0.8	16.1±1.2	15.8±0.2
Visceral fat yield, %	3.26	3.11	3.36	3.32
Slaughter weight, kg	315.2±7.4	304.3±6.9	296.0±10.6	294.8±7.1
Slaughter yield, %	63.4±0.8	61.9±1.0	61.7±0.5	61.9±0.4

It should be noted that the indicators of pre-slaughter live weight in holsteinized breed at 18 months of age varied insignificantly from 476.0 kg to 496.8 kg, and there was no statistically significant differences.
In general, animals in the control group had a superior slaughter qualities, somewhat inferior to them were experimental group I bulls that showed the lowest visceral fat yield - 3.11% (15.3 kg). The research showed the following trend: visceral fat content increases following blood increase in Holstein breed.
For a more complete characterization of meat qualities in experimental bulls, after 24 hours in the refrigerator, dissection of the left halves was performed followed by trimming of the meat by sausage production technology (Table 3).

Table 3 -Morphological composition of experimental bull halves (n = 3)

Parameter	Group							
	Control		Experimental					
			1st		2nd		3d	
	$\bar{X} \pm m$	%	$\bar{X} \pm m$	%	$\bar{X} \pm m$	%	$\bar{X} \pm m$	%
Weight of refrigerated half, kg	147.3 ± 6.10	100	134.7 ± 4.30	100	133.0 ± 6.10	100	129.0 ± 6.90	100
Meat weight, kg	109.7 ± 5.28	74.47	100.4 ± 3.63	74.54	98.8 ± 6.07	74.28	95.3 ± 4.86	73.88
Premium meat, kg	23.2 ± 1.67	15.77	19.6 ± 0.70	14.55	19.8 ± 1.63	14.89	19.6 ± 0.91	15.19
I grade meat, kg	43.3 ± 2.73	29.40	38.1 ± 1.10	28.29	41.3 ± 1.39	31.05	37.0 ± 1.52	28.68
II grade meat, kg	43.2 ± 1.42	29.30	42.7 ± 1.88	31.70	37.7 ± 3.38	28.34	38.7 ± 2.91	30.0
Bones weight, kg	30.3 ± 1.67	20.57	26.2 ± 0.44	19.45	26.0 ± 0.09	19.57	26.7 ± 1.20	20.7
Tendons weight, kg	3.7 ± 0.47	2.46	3.5 ± 0.09	2.60	3.8 ± 0.23	2.84	3.2 ± 0.17	2.46
Bones and tendons weight, kg	34.0 ± 1.43	23.08	29.7 ± 0.46	22.05	29.8 ± 0.15	22.41	29.8 ± 1.36	23.12
Weight of fat, kg	3.6 ± 0.60	2.50	4.6 ± 0.59	3.41	4.4 ± 0.07	3.31	3.8 ± 1.01	3.00
Fleshing index	3.63 ± 0.07		3.83 ± 0.12		3.80 ± 0.20		3.56 ± 0.03	
Fat, points	4.8		4.6		4.6		4.4	

Investigations of morphological structure of halves showed that in crossbred bulls except experimental group I the absolute meat weight and its yield decreased with blood increase compared to the control group by 9.3-14.4 kg (8.5-13.1%) and 0.3-0.6%. The carcasses of animals in the control group contained more bones (by 3.6-4.3 kg) and tendons (by 0.1-0.5 kg) due to greater weight. The relative mass of bones and tendons was also somewhat higher (0.7-1.0%) than that of peers in other groups. Premium meat yield was 0.6-1.2% (3.4-3.6 kg) higher respectively. There was 2.0-6.3 kg more I grade meat in halves of bulls in control group. II grade meat yield in halves of bulls in control group was 1.1% greater than in II experimental group but 2.4% less than in I and 0.7% less than in III. Fleshing index (the ratio of edible parts of the carcass to the bone mass) in animals was practically the same and varied from 3.56±0.03 to 3.83±0.12.

For the analysis of physical and chemical properties of meat we examined the longissimus dorsi at the 9-12 thoracic vertebrae. We also investigated the content of moisture in meat, pH and water retention capacity (Tables 4, 5). Water retention capacity is one of the most important indicators of the quality of meat, its juiciness and appearance.

In our experiment we haven't found significant differences in water retention capacity and pH (pH – 6.3-6.5, water holding capacity – 62.4-68.1%).

Table 4 - Chemical composition of meat in the longissimus dorsi, % (n = 3)

Parameter	Group			
	Control	Experimental		
		1st	2nd	3d
Water	75.99±0.32	76.18±1.09	75.65±0.40	74.90±0.42
Dry basis	24.01±0.32	23.82±1.09	24.35±0.40	25.10±0.42
Crude protein	21.85±0.23	21.33±1.05	21.75±0.41	22.34±0.35
Crude fat	1.33±0.27	1.71±0.24	1.48±0.08	1.98±0.12
Ash	0.82±0.08	0.78±0.18	1.12±0.06	0.78±0.04
Calorific capacity of 1 kg meat, kcal	1020	1034	1029	1100

. Table 5 - Processing characteristics of meat in the longissimus dorsi (n = 3)

Parameter	Group			
	Control	Experimental		
		1st	2nd	3d
Meat water capacity, %	62.4±2.52	65.3±1.93	68.1±2.21	65.5±2.28
pH	6.3±0.04	6.5±0.04	6.4±0.09	6.5±0.15
Boiled meat yield, %	59.0±2.70	62.9±2.41	59.3±1.03	65.0±1.30

Analysis of the data on the chemical composition of meat in the longissimus dorsi shows that no significant differences between experimental animals and control groups were observed. The protein content was high enough and ranged from 21.33% in the animals of experimental group I to 22.34% in the experimental group III.

The highest amount of fat contained in the meat of bulls in experimental groups (1.48-1.98%) and the lowest in the control group (1.33%). Experimental and control groups also did not differ significantly by meat calorific capacity (1020-1100 kcal).

Conclusion

Meat qualities of holsteinized bulls of $^{3}/_{4}$ H$^{1}/_{4}$BV genotype were higher than in animals of other genotypes ($^{1}/_{2}$H$^{1}/_{2}$BV, $^{5}/_{8}$H$^{3}/_{8}$BV and $^{7}/_{8}$H$^{1}/_{8}$BV). Thus, the control group animals had pre-slaughter live weight of 496.8 kg; fresh carcass weight of 299.0 kg; slaughter yield of 63.4%; internal fat content of 16.2 kg, which is higher than in bulls of other groups, respectively: on 5,6-20,8 kg (1.1 – 4.2 %); 10,0 – 20,0 kg (3.3 – 6.7 %); 1.5 – 1.7 % and 0.1 – 0.9 kg (0.6 – 5.6 %). Morphological composition of halves showed that, at an increase in the proportion of Holstein blood in the bulls genotype up to 62.5 and 87.5%, the absolute mass of the meat and its yield decreased slightly in comparison with the bulls of genotype $^{3}/_{4}$H$^{1}/_{4}$BV by 9.3 – 4.4 kg (8.5 – 13.1 %) and 0.3 – 0.6 %. No significant difference in chemical composition of meat was noted.

The research on the comparative study of beef production in black-variegated bulls of different genotypes of Holstein breed under the same conditions of feeding and management suggests that it is more efficient to fatten animals of $^{1}/_{2}$H$^{1}/_{2}$BV and $^{3}/_{4}$ H$^{1}/_{4}$BV genotypes as so as at the same feeding level they have greater live weight, relative weight of meat in the carcass and the fleshing index than their peers ($^{5}/_{8}$H$^{3}/_{8}$BV and $^{7}/_{8}$H$^{1}/_{8}$BV genotypes).

Thus, the study of fattening and meat qualities in bulls of different genotypes of Holstein breed showed that breeding the desired type of animals ($^{3}/_{4}$H $^{1}/_{4}$BV-control) achieved the best results in meat production, and by a number of indicators, such as live weight, average daily gain, fresh and chilled carcass weight and weight of meat and premium meat animals in control group outperform their peers in the experimental groups.

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Original scientific paper
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ASSESSMENT OF THE CARDIOVASCULAR SYSTEM IN SHEEP

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Abstract

It is considered that various changes in the body are caused by diseases that affect the operation of all systems and organs, including the myocardium. The difficulty in detecting heart disease makes this question even more relevant. Diagnostics of the sheep cardiovascular system is also of a specific scientific interest. This question has some difficulties connected with the anatomic features. In the scientific literature this issue is not described well enough.

Receiving quality electrocardiographic curve in sheep largely depends on the method of recording electrocardiogram. Therefore, in our work we paid great attention to the method of the ECG recording in sheep. We considered electrocardiographic changes in sheep of different age and with certain diseases. We also paid attention to the peculiarities of radiographic anatomy of the chest cavity in sheep. Radiography provides valuable data about the condition of the heart and large vessels. That combined with electrocardiographic and ultrasound assessment allows us to conduct a comprehensive assessment of the cardiovascular system in sheep.

Key words: *radiography, electrocardiography, instrumental diagnostics, sheep, goats.*

Introduction

Sheep breeding is one of the important branches of agricultural production in Russia. In recent years it has been gaining in economic importance. Periodic outbreaks of African swine fever in the Voronezh region have forced the population to develop alternative types of livestock. One of these types is the sheep, which can be used to provide the industry with wool and the population with food. The economic value of sheep is their precocity and rapid ability to reproduce.

The analysis of some scientific data [Kotarev V.I., Aristov A.V., Lopatin V.T., Kudinova N.A., Pronina E.A., Capkina N.I., 2014; Parshin P.A., Nikulin I.A., Shumilin Y.A., 2007] shows that the heart condition is often a reflection of the whole organism state. Meat and wool production of sheep depends on the stable operation of the cardiovascular system, therefore, timely detection of violations in this system is the key to successful prevention and treatment. It is considered that various changes in the body are caused by diseases that affect the operation of all systems and organs, including the heart. The difficulty in detecting heart disease makes this question even more relevant. Diagnostics of the sheep cardiovascular system, conducted by radiography, allows you to simultaneously assess the condition of the lungs and bronchi, which is also of particular scientific and practical interest. The solution of these problems has some difficulties connected with the anatomic features. In the scientific literature, they are not described well enough.

In connection with the above data, our goal was to assess the status of the cardiovascular system in sheep with clinical research methods that are available for practical use.

Materials and methods

The work was done in Voronezh State Agricultural University, faculty of Veterinary Medicine and Animal Husbandry Technology in 2015. The study was carried out on clinically healthy sheep Kuchugurovskaya breed (n=13) kept in the vivarium and facilities of the Department of Therapy and Pharmacology. Clinical study of the sheep was carried out according to conventional veterinary plan, taking into account species specificity in

accordance with the recommendations of some authors [Kotarev V.I., Aristov A.V., Lopatin V.T., Kudinova N.A., Pronina E.A., Capkina N.I., 2014; Kotarev V.I., Kryuchenkova N.V., 2003; Nikulin I.A., Shumilin Y.A., 2009; Parshin P.A., Nikulin I.A., Shumilin Y.A., 2007; Yerohin A.I., Kotarev V.I.., Yerohin S.A., 2014]. Special attention was paid to the cardiovascular system. The electrocardiogram was recorded with a three-channel electrocardiograph CARDIPIA 200. The electrodes used clamps of "crocodile" type. For best contact the skin and fur on the places of fixation of the electrodes were treated with electrode contact gel. ECG was recorded by system sagittal derivations suggested by M. P. Roshevskiy with a record of three bipolar body leads and reinforced Goldberg. X-rays of the lungs were performed on a portable x-ray machine DIG-360 using blue sensitive x-ray film and the intensifying screens on the basis of calcium tungstate. Photochemical processing of the films was carried out in the installation manual with reagents company Fudgi.

Results and discussion

A widely used method of studying the function of the heart is the electrocardiogram, which allows to study the functional manifestation of pathological processes affecting the heart, in particular breaches of its electrical activity. On this basis it is possible to develop evidence-based methods of diagnosis of heart disease that is of great importance to practice veterinary medicine. However, many issues of clinical electrocardiography, especially sheep one, are still open.

Receiving quality electrocardiographic curve in sheep and goats largely depends on the method of recording ECG. Therefore, in our work we paid great attention to the method of the ECG recording in sheep. Today, many scientists who study the condition of the heart in hoofed animals, consider theoretically correct and reasonable to carry out the ECG recording in sheep and goats in the sagittal leads, not leads from the limbs, as in humans and carnivores.

For the research a group of clinically healthy sheep Kuchugurovskaya breed (n=13) aged from six months to ten years were taken. We recorded ECG in ewes on the sagittal lead system, which is shared by all ungulates. It allows you to register a bipolar body abduction in the sagittal plane with the electrodes: 1) cranial part of the sternum; 2) the midpoint of the line connecting the caudal angles of the right and left scapulas; 3) the point of intersection of the perpendicular from the 13th thoracic vertebra, with the white line of the abdomen (figure 1). The fourth electrode is the ground electrode and is not involved in the recording of ECG leads, so it can be attached anywhere on the animal's body, at some distance from the 1, 2 and 3 electrodes. Usually we put it in the knee crease.

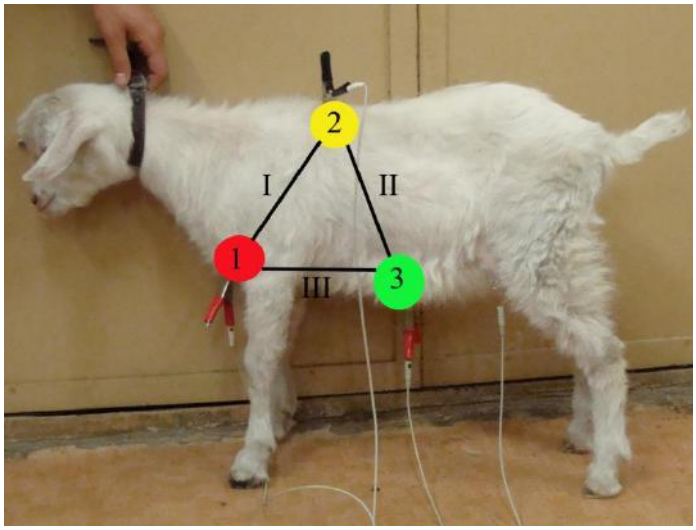


Figure 1. Scheme of electrodes during registration sagittal ECG leads in sheep and goats.

1 – the electrode with the red tip,
2 – the electrode with the yellow tip,
3 – the electrode with the green tip.

I – the lead ECG,
II – the lead ECG,
III – the lead ECG.

The choice of the speed of the paper

partly depends on the heart rate of examined animals. When working with sheep and goats, we recommend you to set the speed of paper movement 50 mm/s. In our opinion this speed allows you to get a good quality recording of cardiac cycles. Amplitude measurement of teeth were performed on unfiltered records because the filter reduces the amplitude of teeth.

In table 1 there are results of a normal electrocardiogram sheep Kuchugurovskaya breed. In the group there were animals (n=10) aged from six months to four years. The basic form of the ECG of the sheep of this breed registered by the system sagittal leads has the following characteristics. In the second lead, which is considered the main, there is a positive atrial prong P value of 0,18±0,01 mV. The initial activity of the ventricles of the heart in 50% of animals presented is Q - 0,54±0,12 mV. The R wave has a small value of about 0,11±0,01 mV. 60% of sheep for R-wave should be deep prong S value of 0,47±0,1 mV. The T wave in the investigated animals is very variable: so 70% of it is positive – 0,21±0,03 mV, in 10% of it is negative – - 0,2 mV, and in 20% of it is two-phase – -/+0,13±0,03 mV.

Table 1. Indicators of normal ECG in sheep Kuchugurovskaya breed (n=10).

Prong	The size of prongs, expressed in mV				
	I the lead		II the lead		III the lead
P	0,09±0,01		0,18±0,01		0,1
Q	80%	0,22±0,04	50%	0,54±0,12	-
	20%	-	50%	-	
R	0,23±0,03		0,11±0,01		0,08±0,01
S	-		60%	0,47±0,1	0,51±0,04
			40%	-	
T	70%	-0,16±0,03	70%	0,21±0,03	0,21±0,03
	20%	0,13±0,03	20%	-/+0,13±0,03	
	10%	-/+0,1	10%	-0,2	

Duration of basic ECG intervals were: PQ – 0,1±0,004 seconds, the wave P – 0,04 seconds, QRS – 0,04 seconds, QT was 0,26±0,01 seconds, and RR – 0,49±0,02 seconds. On the basis of the intervals duration, we can determine the systolic indicator. It shows the amount of electric systole, expressed as a percentage of the total duration of the cardiac cycle. And in our animals the value of systolic indicator is equal to 55%±2%. Knowing the duration of the RR interval, we can accurately identify the number of heart beats. It is 126±6 beats per minute.

The position of the ST segment in healthy animals corresponds to the position of the segment TP (the true isopotential line), i.e. they are located on the same level. ST segment elevation up or down from the true isopotential lines will indicate a violation of the coronary circulation. The ST segment was located at the level of the isopotential lines at all the sheep which we examined. Figure 2 shows a typical electrocardiogram for this group of sheep, which is typical for healthy animals.

The characteristic signs of cardiomyopathy were observed on ECG in three sheep. The electrocardiogram of one of them is presented in figure 3. There was a significant change of the QRS complex in the first lead. It changed its polarity and had splitting on the top of the R-wave. In the second lead QRS complex took the form of the letter W, with the presence of prongs Q, R, S small amplitude. At the same time the amplitude of the wave T was well above the norm, and most importantly it was several times greater than the highest wave of the QRS complex. Systolic indicator increased to 67%.

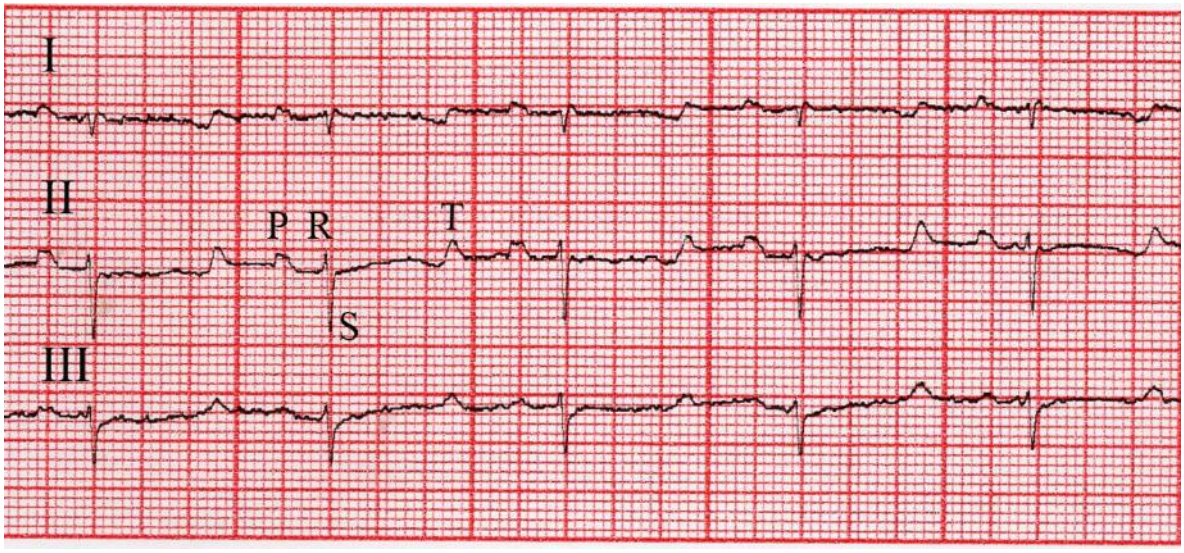


Figure 2. Electrocardiogram of the sheep №8 at the age of 3 years recorded by the system sagittal leads. 1 mV=10mm. The speed of the tape is 50 mm/sec. I, II, III - ECG leads. P, R, S, T – the prongs of the second lead.

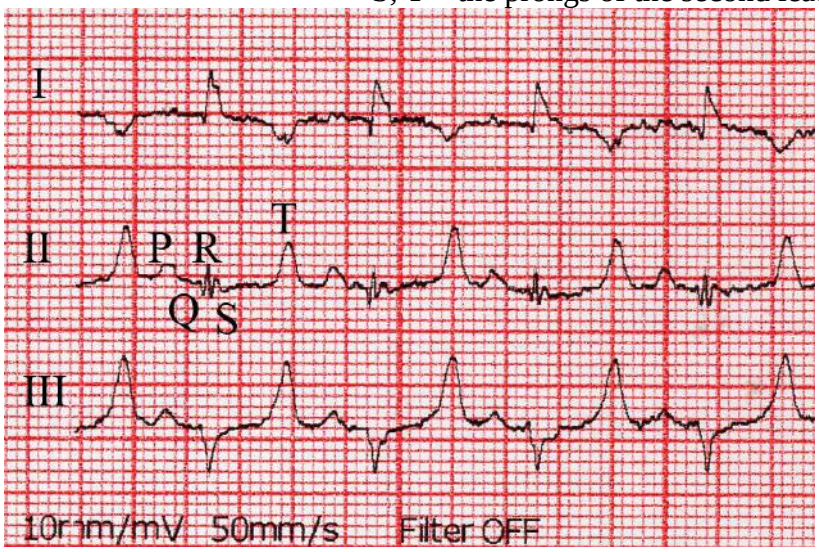
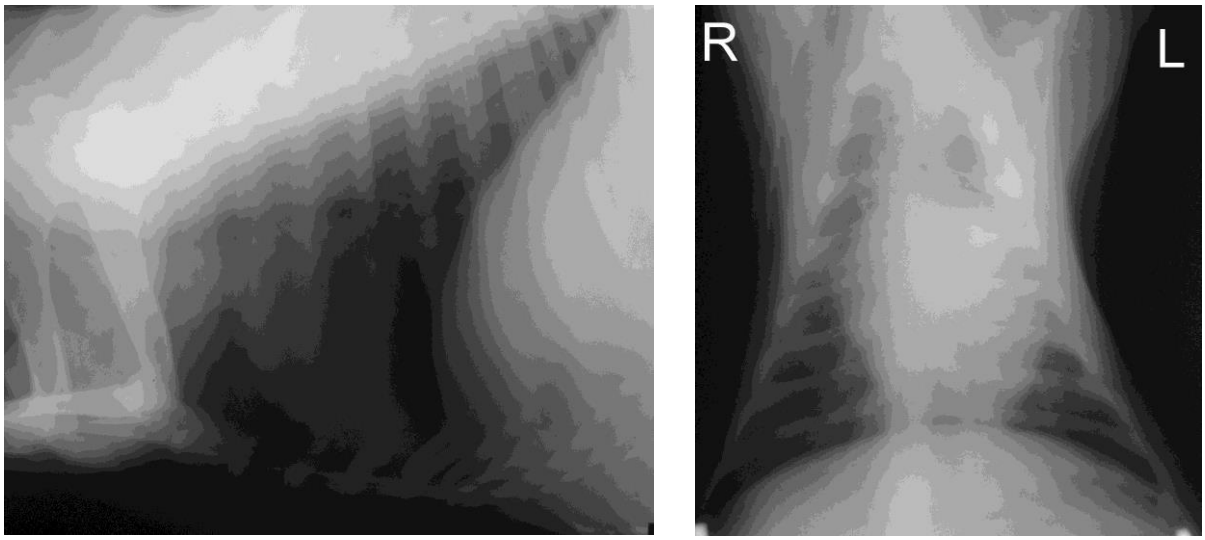


Figure 3. Electrocardiogram of the sheep №12 at the age of 10 years with symptoms of cardiomyopathy. 1 mV=10mm. The speed of the tape is 50 mm/sec. I, II, III - ECG leads. P, R, S, T – the prongs of the second lead.

X-raying the chest in sheep we gave preference to the right lateral recumbent position, as this position is a more typical position of the heart, in this case between the heart and the lower chest wall there are more air lung fields, which enables the study of the elements of the heart and enhances the contrast. The diaphragm does not preclude consideration of the caudal lung fields.

When lying the animal tried to take the front legs forward and lock them to avoid the superposition of the main mass of the triceps on the cranial lobe of the lung.

The most important diagnostic decision is to determine whether the chest is characterized by an x-ray as normal or pathologically altered. To do this we identified key attributes of normal roentgen anatomy of the thorax in sheep (figure 4a, 4b).



a b

Figure 4. Chest x-ray, sheep aged 3 years. a – right lateral projection of the chest, b – ventrodorsal projection of the chest.

The trachea normally is rejected from the thoracic spine at an angle of approximately 40°, it is not visible in the direct projection because of the superposition with the spine.
The silhouette of the heart is egg-shaped with the base of the dorsal and ventral tip. The height of the heart from base to tip is about 2/3 of the height from the size of the chest cavity at the same level. The silhouette of the heart takes about three intercostal spaces laterally. In the direct projection the widest part of the heart is about half, the width of the chest measured at the level of the dome of the diaphragm. In the direct projection of the apex of the heart it is significantly shifted to the left from the middle line.
Caudal Vena cava enters the thorax through the diaphragm and tends cranio-ventral to the caudal border of the heart. Normally its sides are parallel. Cranial Vena cava is composed of the cranial mediastinum and is suitable at a right angle to the cranial border of the heart (figure 4a).
Lung fields are normal to the x-ray transparent in their background pulmonary vessels and the bronchial tree are all visible. The vessels of the lungs come in pairs: artery and vein, the branches of which are directed to the periphery of the lobes of the lung. Arteries and veins are normal, of similar diameter and are on each side of the bronchus. Arteries are dorsal and ventral veins. In the direct projection of the pulmonary vessels may be better seen in the caudal part of the thorax just before the liver.

Conclusion

Sagittal ECG leads applied to sheep meet all the requirements of the theory of electrocardiography for system leads. The shape of the electrocardiographic curve in the sagittal leads is constant when the right position of the body and forelimbs is found. We described electrocardiographic and radiographic indicators characterizing the state of the heart and thoracic cavity organs in sheep, which allow in the future to conduct evaluation the state of these organs in sheep. Knowing the criteria of the normal state different pathological changes can be distinguished. The description of the condition of the heart and lungs in sheep in different pathological processes such as pneumonia, ketosis and other we believe is important to improve diagnostic capabilities and performances of timely diagnosis. There is some prospect of further studies. Thus electrocardiography and radiography can find practical application in the diagnostics of pathology of the cardiovascular system and the lungs in sheep.

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Original scientific paper
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SEROEPIZOOTOLOGY MONITORING OF CYCTIC ECHINOCOCCOSIS IN SHEEP WITH ANTIGENES OF PROTOSKOLEXES OF ECHINOCOCCUS GRANULOSUS (L) AND ECHINOCOCCUS MULTILOCULARIS (L)

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Abstract

Results of serological monitoring of echinococcosis in sheep using ELISA test with excretory-secretory antigens of *Echinococcus granulosus* (l) and *Echinococcus multilocularis* (l) protoscolexes were represented. 58 serum samples of slaughtered sheep with diagnosed parasitological status were tested including 23 from animals with cystic echinococcosis, 24 – without visible changes and 11 – with focal undiagnostic damages in livers. The results of our assay showed that antigens of the both types of echinococcus can be used in serologic monitoring of cystic echinococcosis of sheep. Sensibility of ELISA with *E. granulosus* (l) protoscolexes antigens was 74.3% and with similar antigen of *E. multilocularis* (l) was 73.9% respectively.

Key words: *echinococcosis, antigens, seroepizootology, sheep.*

Introduction

Cystic hidatid disease of sheep belongs to the widespread parasitic zoonoses causing significant economic damage and is dangerous for people. There are many publications about huge losses of animals caused by cystic echinococcosis in the Southern Federal Region where at some farms the extensity of infection exceeds 35-40%. Analysis of the data of slaughtered animals is trustworthy criterion for evaluation of epizootic situation. However in larval cestodoses including cystic echinococcosis the results of serologic studies allowing to conclude (on basis of the received information) about the activity of epizootic process can by an effective element for monitoring of epizootic situation. Considering all this we carried out serologic monitoring of cystic echinococcosis by analyzing serums serums of the slaughtered sheep from farms of Kabardino-Balkaria Republic.

Materials and methods

Serologic analysis was carried out by ELISA, using three-day products of metabolism of *Echinococcus granulosus* (l) and *Echinococcus multilocularis* (l) protoscolexes received by cultivation in RPMI-1640 with 2 ml of glutamine, 8 mg of gentamycin (4%) and 0.5ml of amphothericyn B as antigen. The viability of cultivating protoscolexes was maintained for 3 days in CO_2 incubator “Heraeus” at 37^o C. Serum samples from slaughtered sheep were taken from farms in Kabardino-Balkaria Republic. Helminthological status was determined by investigation of liver, lungs and other parenchymal organs. In total 58 serum samples from sheep were taken including 23 from animals with larval echinococcosis, 24 without visible parasitic changes and 11 - from animals with focal damages in livers. Previously using reference positive and negative control serums we determined an optimal concentration of excretory-secretory antigens for sensibilisation of solid phase and degree of serum solution and titre of conjugate for which rabbit antibodies affine cleared specific to immunoglobulines of sheep, marked peroxidase (“IMTEK”) were used.

Results and discussion

Previous investigations with reference positive and negative control serums by ELISA method showed that the optimal concentrations of excretory-secretory antigens from protoscolexes of *E. granulosus* (l) and *E. multilocularis* (l) for sensibilisation of solid phase were 9 and 11 mkg/ml, respectively. Serum solution was 1:100 and titer of conjugate was 1:6000.

Results of serological monitoring showed that out of 23 serum samples from echonococcus infected animals positive reaction was registered in 18 cases. Therefore, test sensitivity was 78,2% (table 1).

Table 1. Assessment of sensitivity and specificity of immunosorbent assay (ELISA) with 3-day excretory-secretory antigenes of protoscolexes of *Echinococcus granulosus* at echinococcosis of sheep

Serum samples	Number of tests	Results				
		Positive	Doubtful	Negative	Sensibility, %	Spesifisity, %
Larval echinococcosis	23	18	1	4	78,2	74,3
Focal damages in liver	11	5	1	5		
Absence of damages	24	3	-	21		

Positive results were in 17 samples from 23 in similar tests with excretory-secretory antigen from *E. multilocularis* (l) protoscolexes and the same serum samples that corresponded to sensibility of test as 73.9% (table 2). Specificity of assay after reaction with 35 serum samples from sheep with focal damages in liver and without visible damages in livers was 74.3% using homologous antigen and 77.1% using heterological one (tables 1, 2).

The false positive result in the first case was registered in 9 tests, in the second – in 8, and mainly there were serums of sheep with focal changes in liver of an unknown etiology.

Proceeding from the obtained data, we concluded that the both antigen preparations can be used in serologic monitoring of cystic echinococcosis in sheep that is confirmed by rather high sensitivity and specificity of the immunotest characterizing activity of epizootologic process of echinococcosis of sheep in this region.

Carrying out seroepizootologic monitoring of cystic echinococcosis in sheep with use of serums of animals with the known helmintology status and antigene preparations from protoscolexes of *E. granulosus* (l) and *E. multilocularis* (l) confirmed significant antigene relationship of these species of parasites and possibility of their interchangeability in the antigene relation; though there are data about presence of species-specific antigen components in each of them.

According to Ito et. al. for *E. multilocularis* (8) that is protein-antigen component with molecular weigh 18 кДа and for *E. granulosus* – 38 кДа.

Table 2. Assessment of sensitivity and specificity of immunosorbent assay (ELISA) with 3-day ekscretory-secretory antigens of protoscolexes of *Echinococcus multilocularis* at echinococcosis of sheep

Serum samples	Number of tests	Results				
		Positive	Doubtful	Negative	Sensibility, %	Spesifisity, %
Larval echinococcosis	23	17	1	5	73,9	77,1
Focal damages in liver	11	3	2	6		
Absence of damages	24	4	-	21		

Despite it, *Echinococcus multilocularis* (l) antigens were successfully used as diagnostical antigens at cystic echinococcosis in humans (7, 9). Potentially important diagnostic marker of cystic echinococcosis according to Zhang and Mc Manus (11), the component with molecular weight 38 kd, which, as well as component with molecular weight 25 kd, are a part of AG 5 representing the lipoprotein of cystic *E. granulosus* liquid containing subunits within 56-70 kd which at an electrophoresis in poliakrylamid gel with dodecylsulfat sodium dissociate on two components 25 and 38 of a kd.

However, earlier Feng et al. (5,6) reported that two components with molecular weight 52 и 38 кДа were registered in excrets and secrets of germinative cells and cystic liquid of E. granulosus, and the component with molecular weight 52 кДа showed highest specificity in immunological reactions. That dissociation maybe is connected with infective process as there are data that immune response to cestode infection is characterized by development of antibodies of various specificity, for which identification the diagnostic antigene containing molecules to antigene determinants for all synthesizable antibodies at all stages of development of a parasite is necessary.

Indirect confirmation about presence of antibodies with different specificity in serums of infected sheep is data of Ris et al. (10), which increased sensitivity and specificity of ELISA at echinococcosis to 80% at simultaneous holding of reaction with two polysaccharide antigenes of parasite – from protoscolexes and membrane *E. granulosus* cysts. That fact allowed to differentiate cross false positive responces with serums of the sheep infected with *T. hydatigena* (l) and *T. ovis* (l) more accurately.

Conclusion

The analysis of the results of seroepizootological monitoring at cystic echinococcosis in sheep with *E. granulosus* (l) and *E. multilocularis* (l) protoscolexes antigens gives the grounds to make the conclusion, first, about possibility of usage of antigene preparations of both types of echinococs in similar researches, and secondly to assess an epizootologic situation on this helminthosis on sensitivity and specificity of immunoreaction.

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CHARACTERIZATION OF PROTEIN FRACTION IN ENSILED APPLE POMACE TREATED WITH DIFFERENT ADDITIVES

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Abstract

Apple pomace was ensiled without additives and with the addition of dried beet pulp 15%, sunflower meal also 15% and 1% NPN substances, and each of these treatments was with and without inoculant. Certain parameters of suitability of biomass for silage were determined in the starting material, also DM, CP, content of ash, Ca and P. Also, the content of DM, pH, ammonia and soluble nitrogen were determined in the silage. The protein fractions in silage were determined according CNCPS (*Cornel Net Carbohydrate and Protein System*) system as follows: PA, PC and three protein fractions PB (PB_1, PB_2 and PB_3). Apple pomace is extremely suitable for ensiling, it has the most favourable WSC/BC ratio and can be a supplement used for ensiling of biomass that is difficult to ensile. Adding dry feeds such as dry sugar beet pulp and sunflower meal in the amount of 15% increases the content of DM. Sunflower meal provides the highest value of CP (227.5 gkg^{-1}DM) and PB_2 fraction in silage, while adding NPN substances (Benural S) provides the highest value of PA fraction (525.9 gkg^{-1}CP). Application of inoculants has contributed to reducing the PC fraction (214.64:239.47 gkg^{-1}CP), i.e. contributed to the increase of digestibility or nutritive value of silage. High and favourable WSC/BC ratio provided a slightly higher acidity (pH value 3.40 in the control silage, to 3.77 in the apple pomace silage + sunflower meal). The process of protein degradation in the silage expressed through ammonia and soluble nitrogen relative to the total nitrogen in the silage was low, so, the maximum points, i.e. an excellent score for all silage treatments by methods of Zelter and Weissbach and Hoing were awarded. The parameters of biomass suitability for ensilaging were determined in the starting material. Also were determined DM, CP, ash content, Ca and P.

Keywords: *apple pomace, sunflower meal, NPN substance, protein fractions, quality of silage.*

Introduction

The problem of the deficiency of animal feed for ruminants, especially in years of drought can be mitigated by using certain related products to the food industry, even in nutrition of dairy cows (Ghoreishi et al. 2007). Some by-products may have other uses as raw material for the production of compost, for the production of alcohol (Chatanta et al., 2008), but the best way of utilization is their preparation and conservation so that they can be used as part of the ruminants' diet.

Apple pomace is the residue remaining after pressing of apples and removing the juice and is composed mainly of fruit skin, seeds, and the rest of the juice. Fresh apple pommace, contains a low level of DM (Pavličević et al., 1988). In ruminants' diet fresh, ensiled and dehydrated apple pomace can be used. The use of fresh pomace in animal nutrition in the long run is not possible due to the rapid fermentation or spoilage. Dried apple and grape pomace has been previously used in ruminant nutrition, primarily in cattle fattening (Zeremski, 1982, Nikolić et al., 1986), however, presently such nutrition is not economically viable.

Taking into consideration that apple pomace is poor in protein (Abdollahzadeh et al., 2010), and rich in water soluble carbohydrates, it is necessary to apply a method of increasing the content of the CP, in order to optimize ruminant nutrition. One of the simple and extremely inexpensive methods is the addition of a suitable source of non-protein nitrogen in the silo mass or diet. Symbiotic bacteria in the rumen will, on the basis of added nitrogen, synthesize their own proteins of high biological value, which will be digested and used in the small intestine of ruminants together with dietary proteins (Đorđević and Dinić, 2011).

One of the potential problems related to the utility value of silage from apple pomace could be insufficient aerobic stability. The main reason for this is very broad relationship of water soluble carbohydrates (WSC) and the buffering capacity (BC), and any potential improper utilization and exclusion method of silage. Therefore, the use of the bacterial inoculant is planned, which in addition to homofermentative also contains heterofermentative bacteria of lactic acid fermentation.

The aim of this paper is to examine the possibility of ensiling apple pomace by adding NPN substances, sunflower meal, dried beet pulp to increase the dry matter content, determine the protein fractions according to CNCPS and increase the anaerobic stability of the silage.

Material and methods

Ensiling of apple pomace was performed on April 3rd 2013, in experimental containers holding 130 dm^3, in three replications. Study treatments in the present research of the apple pomace silage were: A_1 - ensiled apple pomace 100% participation (a_1b_1 - apple pomace without additives; a_1b_2 - apple pomace with inoculant; A_2 - apple pomace 85% + 15% dried beet pulp (a_2 b_1 - without additives; a_2b_2 - with inoculant); A_3 apple pomace 85% + 15% sunflower meal (a_3 b_1 - without additives; a_3b_2 - with inoculant); A_4 - apple pomace 84% + 15% dried beet pulp + 1% Benural S (a_4b_1 - without additives; a_4b_2 with inoculant).

Apple pomace is characterized by extremely high moisture content, yellow colour and pleasant smell. Dry sugar beet pulp and sunflower meal were purchased commercially and were according to their declaration.

As a NPN substance Benural S was used which contained 42% of urea, 56% of bentonite and 2% of sulphur. In contrast to the pure urea, this product contains the bentonite, which allows slower release of ammonia in the rumen, and more efficient utilization by the microorganisms of the rumen, it binds some gases and toxic substances, and contains certain important elements (K, Na, Mg etc.). The sulphur present in Benural S enables efficient synthesis of some essential amino acids that contain this macro-element (methionine, cysteine).

BioStabil Plus is an inoculant which contains homofermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*) and heterofermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of $5x10^{10}$cfu per gram of product.

Certain parameters of the ensiling suitability of the biomass were determined in the starting material (apple pomace, sunflower meal, dry sugar beet pulp). WSC (water soluble carbohydrates) were estimated colorimetrically by the method of DuBois et al. (1956), and the buffering capacity (BC) according to the method of Weissbach (1967). The following parameters were determined in the apple pomace and silage: content of DM, CP, ash, Ca and P; the content of DM in the silage, the degree of acidity (pH), ammonia and soluble nitrogen.

Protein fractions according CNCPS (*Cornel Net Protein and Carbohydrate System*) were determined by the method Licitra et al. (1996), and fractionated into 5 fractions (Snifen et al., 1992; Fox et al., 2003), as follows: PA fraction of CP – easy soluble proteins that comprise the non-protein nitrogen; PC fractions of CP - completely unavailable/inaccessible CP representing ADICP (*Acid Detergent Insoluble Crude protein*); PB fraction of CP - potentially degradable fraction is the difference between the total crude protein and the sum of soluble and unavailable crude proteins. PB crude protein fraction is further subdivided into:

PB_1 fraction which is rapidly degraded; PB_2 fraction that is of medium degradability and PB_3 fraction which is slowly degraded in the rumen.
The chemical analyses were performed in the laboratory of the Institute of forage crops in Kruševac, according to standard methods (AOAC, 1990). For evaluating the quality of silage - based on ammonia and water soluble nitrogen, two methods were applied - Zelter (Đorđević and Dinić, 2003) and Weissbach-Hoing (1997). The results of chemical analyses were analysed by analysis of variance, and the statistical significance of differences was tested using the LSD test.

Results and Discussion

In order for a biomass to be suitable for ensiling, it should contain as many as possible WSC, and at the same time as low as possible BC. In order to ensure successful lactic acid fermentation and obtain a stable silage without the presence of butyric acid, the WSC/BC ratio should be equal to or higher than 3.0 (Weissbach, 1967), and it is desirable that the level of dry matter in the biomass of roughages is 300 - 400 gkg^{-1}.

The low content of DM was determined in apple pomace - 170 gkg^{-1}, which forced us to use dry sugar beet pulp in order to increase the level of DM. WSC level was extremely high (498.6 gkg^{-1}DM, and the highest in the research so far with very low BC of 6.69 lactic acid (LA) to 100 g^{-1}DM (Table 1), which contributed to the highest WSC/BC ratio of 74.5 suggesting that apple pomace can be a great addition to all biomasses that are difficult to ensile. Dry sugar beet pulp is considerably less favourable in comparison with apple pomace and WSC content in the sunflower meal is a trace, below 1%.

Table 1. Results of the parameters suitability apple pomace and used supplements for silage

The starting material	DM, gkg^{-1}	WSC, gkg^{-1}DM	BC, meq LA $100g^{-1}$ DM	Ratio WSC/BC
Apple pomace	170.0	498.6	6.69	74.5
Dry beet pulp	869.7	28.1	14.1	1.99
Sanflower meal	901.8	8.93	76.9	0.12

Legend: BC - buffering capacity; LA - lactic acid; WSC - water soluble carbohydrates (sugars);

CP content and content of minerals in the starting material are given in Table 2. The lowest concentration of CP in apple pomace can be observed (79.0 g kg^{-1} DM), and the highest in sunflower meal (333.1 g kg^{-1} DM). A similar statement is in regard to mineral substances, sunflower meal contains close to 3.5 times and dry sugar beet pulp 3 times more compared to apple pomace.

Table 2 Concentration of protein and minerals in the starting material, gkg^{-1}DM

Treatments	CP	Ash	Ca	P
Apple pomace	79.0	20.8	2.7	1.3
Dry beet pulp	91.3	64.7	14.3	1.2
Sanflower meal	333.1	73.6	7.1	11.3

Ca concentration is the highest in dry sugar beet pulp, 2 times higher compared to sunflower meal and 5 times higher than in the apple pomace. The concentration of P is almost identical in apple pomace and dry sugar beet pulp, and over 10 times higher in sunflower compared to these feeds (Tab. 2).
The concentration of CP in silages depended on added nutrients. The lowest concentration of CP was established in silages of pure apple pomace - silages A_1 and A_2 (86.99and 89.75g kg^{-1}

DM) (tab. 3). Adding NPN substances to apple pomace in the amount of 1% (silage A_4) contributed to a significant increase in the content of the CP in the silage (140.25 g kg $^{-1}$ DM), and the highest values of CP in silage determined in A_3 to which 15% sunflower meal was added (227.52 g kg $^{-1}$ DM). Statistical analysis showed highly significant differences in the content of the CP between silages A_1 and A_2, on the one hand, and apple pomace silage with the addition of NPN substances (A_4) and sunflower meal (A_3) on the other hand. Statistically significant difference in the content of CP between silages A_3 and A_4 was determined, but no significance was established between silages with and without inoculant.

PA fraction of CP represents the easy soluble proteins that comprise the non-protein nitrogen. Values of PA fraction were in the range of 268.5 to 525.9 g kg $^{-1}$ CP (tab. 3). The highest value was found in silage where the NPN substance was added (Benural S). Statistical significance was established between silage A_3 and other silages, but no significance was established between silages with and without the inoculant in regard to the content of the PA (protein fraction).

Table 3 Protein fractions of apple pomace silage with added nutrients

Tretmani		CP g kg $^{-1}$DM	PA g kg $^{-1}$ CP	PB_1 g kg $^{-1}$ CP	PB_2 g kg $^{-1}$ CP	PB_3 g kg $^{-1}$ CP	PC g kg $^{-1}$ CP
A_1	B_1	86.55c	259.1b	2.27a	299.9b	46.14c	392.59a
	B_2	87.44c	321.7b	0.60a	321.6b	28.20cd	327.90b
A_2	B_1	87.64c	278.8b	0.40a	225.7cd	229.07a	266.03c
	B_2	91.87c	258.1b	0.27a	263.4bc	246.03a	232.20d
A_3	B_1	228.86a	298.8b	2.33a	555.2a	19.47d	124.20f
	B_2	226.17a	274.2b	0.22a	577.6a	25.69cd	122.29f
A_4	B_1	137.59b	499.2a	0.20a	209.4cd	116.20b	175.00e
	B_2	142.91b	552.6a	2.23a	171.4d	97.74b	176.03e
$\overline{X}$ A_1		86.99c	290.4b	1.43a	310.8b	37.17c	360.25a
$\overline{X}$ A_2		89.75c	268.5b	0.33a	244.5c	237.56a	249.12b
$\overline{X}$ A_3		227.52a	286.5b	1.27a	566.4a	22.57c	123.23d
$\overline{X}$ A_4		140.25b	525.9a	1.22a	190.4d	106.98b	175.54c
$\overline{X}$ B_1		135.16a	334.0a	1.30a	322.5a	102.73a	239.47a
$\overline{X}$ B_2		137.10a	351.6a	0.83a	333.5a	99.43a	214.64b

PB_1 fraction of CP in silage that is characterized by rapid degradability, was present in a small amount and no statistical significance between the silage treatments was established (tab. 3). PB_2 fraction of CP is fraction of medium degradation, with the lowest values determined in the silages with NPN substance (190.4 g kg $^{-1}$ CP), and the highest value was found in silages with sunflower meal (566.4 g kg^{-1}CP). Statistical significance was established between silages of all treatments, except for the silages with and without the application of inoculants (table 3). Minimum value of PB_3 fraction of crude protein (22.57 g kg^{-1}CP), which degrades slowly in the rumen, was established in silages A_3 (with the addition of sunflower meal) and in control silages (37.17 gkg^{-1}CP), and the highest value (237.56 gkg^{-1}CP) was found in apple pomace silage with the application of inoculant. PC fraction of crude protein is completely inaccessible crude protein which represents ADICP or CP that remains undegraded after treatment of nutrients in acid detergent. It is interesting that the highest value (360.25 g kg^{-1} CP) was established in silages without additives, not treated with inoculant either. It is supposed that the lactic acid bacteria and their enzymes have contributed to greater degradation of CP and PC fraction values were lower when applying inoculants. The minimum value of the PC fraction (123.23 g kg^{-1}CP), almost three times lower than in the

control silage, was found in silage supplemented with protein feed (sunflower meal). Statistically significant difference between all silages was established, in silage treatment factor A, as well as silage treatment factor B. Application of inoculant provided a lower value of PC fraction by nearly 25 g kg^{-1}CP (tab. 3), i.e. increased digestibility and degradability of CP and created the possibility for increased production of milk and meat.

The biochemical changes in silages

The dry matter content was in the range of 172.5 g kg^{-1} in A_1 silage without added dry feeds to 233.3 g kg^{-1} at the level of factor A_3 (tab. 4). DM levels in silage below 300 g kg^{-1} is considered to be unfavourable for lactic acid fermentation process, due to losses in the form of juice, which appears during ensiling process, and due to prevention of activities of spoilage microorganisms (Зафрен, 1977). A highly significant statistical difference was established between the silages without and with added dry feeds.

Table 4. Results of chemical analysis of the lactic acid fermentation process

Treatments		DM	pH	% in DM silage		% N / ΣN	
				NH_3-N	H_2O-N	NH_3-N	H_2O-N
A_1	B_1	176.7c	3.42cd	0.54c	4.30d	3.90b	31.13c
	B_2	168.3c	3.38d	0.76b	4.17d	5.43a	29.75cd
A_2	B_1	228.0ab	3.48cd	0.37d	3.83d	2.67c	27.35d
	B_2	235.0a	3.52c	0.20e	2.67e	1.38d	18.16e
A_3	B_1	236.0a	3.81a	1.40a	11.33b	3.82b	30.99c
	B_2	230.7ab	3.74ab	1.26a	10.27c	3.49bc	28.37cd
A_4	B_1	235.7a	3.67b	1.26a	11.10b	5.72a	50.42b
	B_2	226.0b	3.51c	1.30a	12.50a	5.66a	54.67a
$\overline{X}$ A_1		**172.5b**	**3.40d**	**0.65b**	**4.23c**	**4.67b**	**30.44b**
$\overline{X}$ A_2		**231.5a**	**3.50c**	**0.29c**	**3.25d**	**2.03d**	**22.76c**
$\overline{X}$ A_3		**233.3a**	**3.77a**	**1.33a**	**10.80b**	**3.66c**	**29.68b**
$\overline{X}$ A_4		**230.8a**	**3.59b**	**1.28a**	**11.80a**	**5.69a**	**52.55a**
$\overline{X}$ B_1		**219.1a**	**3.60a**	**0.89a**	**7.64a**	**4.03a**	**34.97a**
$\overline{X}$ B_2		**215.0a**	**3.54a**	**0.88a**	**7.40a**	**3.99a**	**32.74b**

The degree of acidity of silage is the first indicator of the quality of silage. The highest acidity (pH = 3.40) was found in the silage without added dry feed, and the lowest in silage with sunflower meal (pH = 3.77) (tab. 4). The differences were statistically significant only between silage A_1 on the one hand and other silage treatments. Although the differences were significant in the context of factors in all treatments, methods for evaluating the quality of silage consider as a significant value only difference of above pH 0.20 units. In this study, a significant difference would be between the A_1 and A_3 silages and border significance between the silages A_1 and A_4.

The amount of ammonia nitrogen (NH_3-N/ΣN) is a basic indicator of complete protein degradation. The amount of soluble nitrogen in the silage (H_2O-N/ΣN) also represents a parameter indicating the process of degradation of protein in the silage. Both indicators are expressed in absolute values gkg^{-1}DM and as a % of ammonia nitrogen relative to the total nitrogen. The values in these studies for NH_3-N/ΣN are extremely low, ranging from 2.03% in the silages A_2 to 5.69% in silages A_4. The highest value was expected in silages A_4 since they were supplemented with NPN substance. Differences in these parameters were statistically

significant, but if the silage is evaluated only on the basis of NH_3-N/ΣN, maximum points (great scores) by the method of Zelter (Đorđević and Dinić, 2003) and Weissbach and Hoing (1997) are obtained when the content of NH_3-N/ΣN is up to 10%. Slightly more stringent criterion has the method by Dulphy and Demarqilly (1978), where the content of NH_3-N/ΣN of alfalfa silage to 8.00%, and of other silages to 7.00% of NH_3-N/ΣN receives the maximum points, or excellent score for that parameter.

The values of soluble nitrogen in these studies were slow, and only in one case exceeded 50% H_2O/ΣN in the silages with added NPN substances (tab. 4). The concentration of soluble nitrogen compared to the total is scored the highest if the percentage is 50% or less, which is almost in all treatments except silages with added NPN substances.

Conclusion

Based on the performed research of ensiling apple pomace with the addition of dried beet pulp, sunflower meal and NPN substances, the following can be concluded:

Apple pomace is extremely suitable for silage, has a very favourable WSC/BC ratio and can be used as a supplement for biomass that are difficult to ensile;

Addition of dry feeds (dry sugar beet pulp and sunflower meal) in the amount of 15% increases the content of DM and creates favourable conditions for the process of ensiling;

Sunflower meal in the amount of 15% provided the highest value of CP (227.5 g kg^{-1}DM) and PB_2 fraction in silage, while adding NPN substances (Benural S) in the amount of 1% provided the highest value of PA fraction (525.9 g kg^{-1}CP).

Use of inoculants contributed to reducing the fraction PC (214.64 : 239.47 g kg^{-1}CP), i.e. contributed to the increase of digestibility or nutritive value of silage.

High WSC/BC ratio provided a slightly higher acidity (pH value 3.40 in control silage, to 3.77 apple pomace silage + sunflower meal).

The process of protein degradation in the silage expressed by ammonia and soluble nitrogen relative to total nitrogen in silage was low, resulting in maximum points, i.e. an excellent score for all silage treatments by methods of Zelter and Weissbach and Hoing

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THE EFFECT OF PRO-MIXTURE ON THE QUALITY OF CHICKEN EGGS FOR CONSUMPTION

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Abstract

Pre-mixture Panto L 5/25 is imported component Hl Hamburg – Germany. The experiment was carried on ISA Brown hybrid laying hens, on mini farm. The experiment lasted for 150 days. There were 750 hens in one control group (C) and 740 in the experimental group (E) ,,Agro-PLUS''1% (AK) 1% of pre-mixture was added to fodder in the control group and 5% of the same pre-mixture Panto L 5/25 was added in the experimental group.

It has been confirmed that the quality of eggs is determined by physical and chemical characteristics. To evaluate the quality of eggs in technical way, there are many parameters that have to be considered such as weight of egg, egg length, widths of egg, shape index, shell color, shell cleanliness, yolk color, yolk height, albumen height, yolk weight, white of egg's attitude, Haugh units, shell thickness, shell hardness, egg grade etc.

Many genetic factors influence on the value of the above-mentioned parameters, while para-genetic factors influence on other parameters.

The goal of this research is to obtain scientific data on the impact of premix Panto L 5/25, added to complete fodder mixture for laying hens, on the quality of table eggs.

Keywords: *egg-laying hens, pro-mixture, evaluate the quality of eggs.*

Introduction

The premix is a feed mixture that contains vitamins, minerals, colors, methionine, proteins, lysine, fats and oil, phytases… Antioxidants that inhibit the formation of peroxides, i.e. fat rancidity, stabilizers, binding agents, and agents against congelation, and preservatives, have been added to the premix.

Premixes have a favorable influence on better utilization of the genetic potential of livestock, with the aim to achieve a higher and more cost-effective production. Koljajić et al., (1996), Pavlovski et al., (1996) followed the influence of premixes on the eggshell quality, which would reduce losses during transportation. Researchers Kermanchoi and Golion, (1991) believe that, apart from proteins, mineral substances should also be added, in order to regulate the shell quality. The ratio of mineral substances have been examined by Guinate and Nys 1991), through different particle size, where large particles stayed longer in the gizzard and served as a reserve of some elements that hens used when they did not eat.

Vitamin E relieves stress in laying hens and broilers. Woolford (1994) has found that increased levels of calcium in feed act in a depressive way on feed consumption, while an increased concentration of magnesium acts adversely to the adoption of calcium. The level of calcium of 3.5 - 4% in feed daily is required for the shell formation.

Oviposition, and the time of adding marble (Pavlovski et al., 2005) influence the quality of eggs for consumption.

The manner of keeping hens (floor system or in cages) has influenced the quality of eggs (Pavlovski et al., 1982, Torges et al., 1976).

The knowledge of a range of factors that affect the quality of the eggshell, and the internal egg quality, is essential for the production of high quality eggs (Roberts 2004).

The aim of this work is to obtain scientific data on the influence of Panto L 5/25 premix, added to complete feed mixtures.

Material and methods

The experiment was conducted on a mini farm, in laying hens of Isa Brown hybrid (crossbreed of Leghorn and Rhode Island). The experiment lasted 150 days. The number of hens was 754 in the control group (C) and 740 in the experimental group (E). One percent of premix of our own production (AK) was added to the feed mixture for the control group, and Panto L 5/25, 5% was added to the experimental group. The hens were kept in a cage system, with 4-5 hens per cage. Since the experiment lasted 150 days, the samples were taken at the beginning of the experiment, examination I, in the middle of it, examination II, and at the end of it, examination III.

The following parameters were evaluated:

the weight of fresh eggs, determined by measuring on an electronic scale

egg length and width, measured by a calculator with the accuracy of 1 mm

shape index, length to width ratio, obtained by computation. It is the egg shape, which is calculated by dividing the egg width by egg length, and multiplying by 100. The normal egg shape has the shape index of around 73. The normal index is from 61 to 86.

shell color can be determined visually or by using a refractometer. It is evaluated by points from 1 (light) to 5 (dark). Although the shell color is not an indicator of quality, it is often very important for consumers.

the shell purity, visually determined

USDA class, an American standard

egg yolk color, La Roche range (Vuilleumier, 1969).

egg yolk height (index), the yolk diameter was determined by a micrometer

egg white (albumen) height, determined in the same way as the egg yolk height

Haugh unit (HU), calculated with a calculator, according to Haugh's method (Haugh, 1937), based on the height of thick egg white (H, mm) and the egg mass (W, g)

shell thickness, measured with a micrometer on a piece of shell without the membrane.

Results and discussion

According to the technological classification, this premix (a combination of vitamins and minerals) is very good for feeding laying hens. It is intended for meals containing a higher concentration of proteins (25%) and fats (8%), is kept at a temperature up to 25°C, and is resistant to pelleting.

Evaluations of the quality of the eggs for consumption are in Table 1.

Table 1. Evaluations of the quality of the eggs for consumption from Isa Brown hens

Parametric egg quality characteristics	Average for the full view					
	control (C) group			experimental (E) group		
	n	x	Sd	n	x	Sd
Egg weight (g)	45	68,58	4,03	45	69,54	4,29
Egg width (mm)	45	46,54	0,40	45	46,32	0,54
Length eggs (mm)	45	57,59	0,71	45	57,85	1,01
Shape index (%)	45	75,07	2,06	45	75,38	1,96
Yolk colour (1-15, Roche)	45	11,05	0,89	45	11,18	0,82
Yolk height (mm)	45	18,62	0,88	45	18,98	0,82
Yolk weight (g)	45	16,72	2,24	45	16,72	2,03
Albumen height (mm)	45	8,11	0,93	45	8,35	0,79
Haugh Units (score)	45	85,91	5,60	45	*87,58	4,95
Egg shell thickness (μm)	45	356,87	32,64	45	357,78	37,59

n=number of samples, x=average (mean), Sd= standard deviation,*= exponents are statistically different (P<0.05).

The egg weight during the experiment ranged from 67.51 g - 70.12 g. A statistical significance has been found in the egg mass at the end of examinations II and III, in favor of E group compared to the C group.

The egg class; in the control group, the largest number of eggs was of class B, then class A, and lastly class C. The laying hens of the experimental group had eggs of class AA, then class A, less of class B and the least of class C. Each country has its own rulebook on classifying eggs, and it is mainly based on the measured weight of eggs (Pravilnik o kvalitetu jaja i proizvoda od jaja, 2003 i 2004., eng. Regulation…, 2003 and 2004). According to the quality based on the USDA classification (USDA, 2000), it is necessary to break the egg and observe how much it is spilled on the surface. When the broken egg covers a smaller surface, and when the egg white is firm (min 72 Haugh units), the egg class is better.

The egg width; this feature is inherent, and therefore it is less affected by feed. Larger breeds produce larger eggs. The egg size increases from the time hens begin laying to the 6^{th} month of laying. Averagely for the entire experiment, the egg width for C group was 46.54 mm and 46.32 mm for the E group. A statistical significance has not been found here.

The egg length, as well as the width and size, depend on genetic factors. During the entire experiment, the index value for the egg length was higher in E group by 0.45%.

The egg shape index; this is also a parameter linked to genetic factors, heritability (Supić et al., 2000), and in examination I, a statistical significance between the control and experimental group was found. During the entire experiment, a difference of 0.41% in E group compared to the C group was found.

The yolk color; a statistical significance was not found in this evaluation, but the average for the entire experiment was higher by 1.18% in E group compared to C group. Serbian market values the eggs with more intensive yolk color (10-12 Roche). The yolk color is determined visually by comparing it with the color palette that is in the range. The span of the range is 1-15.

The egg yolk height (index); no statistical significance was found in the evaluation of this parameter, but the average value for E group throughout the entire experiment had a lower level by 1.93% compared to C group. This parameter is determined by the ratio of yolk height and yolk width, and the obtained number is multiplied by 100. Normal values are 32-58%.

The yolk weight; throughout the entire experiment, the indices in both groups were unvarying.

The height of egg whites has an influence on Haugh units. The difference of average egg white height between the examined groups was significant in the third examination.

Averagely for the entire experiment, the relative indicator was 2.96% higher in E group compared to the C group. The high value of Haugh units indicates a high value of egg white quality.

The number of Haugh units in eggs is calculated according to the formula for the height of hard egg white and egg weight. There was a statistically significant difference in the average number of Haugh units of eggs from laying hens in examination I and during the entire experiment between E and C groups (higher in E group by 1.94%).

The thickness of egg shell; a statistical difference was found in the third examination in E group by 0.25% compared to C group, and this characteristic is influenced by genotype.

The egg quality depends on the hen age, genetic traits and meal balance.

The role of evaluating the height of egg whites is very important, and with it, a direct role and the importance of Haugh units. Studies have not achieved results in some relations of the external quality such as the shell thickness. For this reason, according to some authors, some individual elements and vitamins have to be added. Eggs have a certain number of Haugh units, which means that they are of high quality.

Conclusions

From what has been said, we can conclude that, when preparing meals and balancing the composition of the mineral-vitamin and antibiotic supplements, everything has to be carefully done, so that they would be appropriate. Genetic characteristics and technological requirements of provenances for chicken eggs for consumption have to be taken into account. Based on our results, Panto L 5/25 premix has shown economic justification and is recommended for the production of feed mixtures for laying hens. The research has found that there were eggs of AA class in the experimental group, while there were no eggs of this class in the control group.

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VARIABILITY OF WING NERVATURE ANGLES IN HONEYBEES (Apis mellifra carnica Poll) FROM VARIOUS LOCATIONS OF RASINA DISTRICT

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Abstract

Morphometric characteristics of honey bees are used in the taxonomy of honey bees because of that they genetically depend on the race. Chitin nervature of honey bee wings has become a subject of interest to researchers worldwide and in our country. It was observed that these parameters show variability similar to other biometric characteristics. The aim of this paper was to determine the variability of angles of wing nervature of honey bee from the area of Rasina District. The experiment included three sites that are located in the immediate vicinity of Kruševac (Mačkovac, Makrešane and Majdevo). After measurements of selected characteristics, their variability was determined. Eleven angles on the chitinous nervature of front right wing were measured. The highest average values for angles A4 (33.1º), J10 (56.1 º), K19 (7.5 º), L13 (17.2 º) and O26 (41.6 º) were determined on samples from the site Majdevo. In samples from the site Makrešane the highest average values were for the B4 (111.1 º), D7 (99.0 º), E9 (22.4 º), G18 (92.3 º) and J16 (98.1º). The bees from the site Mačkovac had te highest average value for the angle N23 which was 97.6 °. Statistical analysis of data by localities was done and coefficients of variability were determined. The highest variability of angles A4 (7.62), B4 (31.22), D7 (3.40), E9 (7.85), J16 (13.24), K19 (4.02) and N23 (5.40) were found in bees from the site Mačkovac. Honey bees from the site Majdevo showed the greatest variability for angles J10 (8.83) and O26 (6.27).

Keywords: *honey bee, wing nervature, Rasina district*

Introduction

In various areas of the world, different honey bee races evolved under the influence of different environmental factors. These races have their specific biometric features and characteristics that are used in their identification and classification. *Apis mellifera carnica* Pol., also known as domestic carnica or the carniolan grey bee populates the Balkan Peninsula and the valley of the Danube. Area of its distribution is bordered by the Adriatic Sea, the Julian Alps, the Carpathians and the Black Sea. Biometrics is a method that can detect differences within taxa. The first biometric researches studied a few observable and measurable traits (like size and color), but today it is considered that good biometric study should include a greater number of different morphological parameters.

In recent decades, both in the world and in our country, all researches focus on the potential of honey bees from the point of morphometric parameters with the aim of more efficient exploitation of honey bee and its introduction into the selection programs. Considering carniolan honey bee, Ruttner (1988) was among the first scientist to note that different ecotypes can be determined within the race through precise morphometric studies. Elements of external morphology of carnica from Serbia were studied by Krunić (1967), Stojanović (1992), Plužnjikov (1995), Georgijev (2001), Stevanović (2002), Jevtić (2007) and others.

All above mentioned researchers found that there was considerable variation in almost all of observed morphometric traits of honey bees from this area. Variation of some traits is so high

that it differentiates certain populations (local population) in relation to other colonies from neighboring areas. These differences are primarily conditioned by the specificity of relief and eco-climatic conditions on the territory of Serbia. Alpatov (1929) and many authors after him showed that the environment can have a major impact on the morphological traits of honey bee.

The aim of this study was to determine the variability of angles of wing nervature in the honey bees from very narrow geographical area which is one part of Rasina region (an area of (from Kruševac to Stalać).

Material and methods

In this paper, the honey bees from local honey bee populations from areas of central Serbia were analyzed. The study was conducted in 2012. Colonies were taken from the apiaries from three different sites near Mačkovac, Makrešane and Majdevo. Five colonies were selected from each site, and from each colony 30 bees were sampled. Samples of bees were an indigenous source material.

After the sampling, the bees were kept in 96% ethyl alcohol and stored in a refrigerator at a temperature of from -4°C until measurement. The eleven angles of chitinous nervature of front right wing were measured: A4, B4, D7, E9, G18, J10, J16, K19, L13, N23 and O26. Morphometric measurements of angles were performed by standard method by Ruttner et al. (1978).

Temporary anatomical preparations of the wings were made, scanned in high resolution of 9600 dpi and then processed by computer software Motic Images Plus 2.0.

After measuring, descriptive statistics was performed for all parameters and standard deviation and coefficients of variation were calculated.

Results and discussion

Angle A4 had the average value of 31.43° and the highest value was in bees from the site Majdevo, while the lowest was in bees from Mačkovac (Table 1). The highest variability within the same site was found in bees from Mačkovac (CV=7.63). By the analysis of variance, it was found that the site had a statistically significant effect on the expression of this trait (level of significance P=0.05).

The average value of the angle B4 was 103.63°, the highest value was measured in Makrešane (111.1°) and the lowest in Mačkovac (93.9°). Analysis of variance, as a single factor experiment, showed that location had very significant impact on the expression of this trait on the level of probability P=0.01. Testing the difference (LSD test) showed that the honey bees from all three sites differed very significant in the size of the angle B4. Obtained results for the honey bees from the site Makršane are similar to the results of Maul and Hähnle (1994) and Stojanović (2002), but the other two sites differ considerably from those data.

Angle D7 in the analyzed samples of honey bees had a value of 98.33°, and very little variation considering the sites. This angle had the lowest coefficient of variation in total and by sites. The differences in the mean values were not statistically significant. The results are consistent with the results Maul and Hähnle (1994), but are slightly higher than in the research Stojanović (2002) and Nedic et al., (2011), and slightly lower compared to the results obtained in research Mladenovic and Simeonova (2010).

Table 1. Angles of wing nervature (°) in colonies from three different locations in Rasina region in Serbia

Site		A4	B4	D7	E9	G18	J10	J16	K19	L13	N23	O26
Makrešane	$\overline{X}$	30.8	111.1	99	22.4	92.3	54.3	98.1	74.8	14.1	96.7	40.6
	x_{min}	27	104	96	21	91	49	91	70	12	91	35
	x_{max}	33	120	102	24	98	59	104	77	17	102	44
	SD	2.29	4.89	1.76	1.26	2.12	3.28	4.89	2.09	1.79	4.11	2.55
	CV	7.47	4.39	1.78	5.64	2.28	6.02	4.98	2.80	12.71	4.25	6.27
Majdevo	$\overline{X}$	33.1	105.9	98	22.3	90.7	56.1	97.1	79.5	17.2	93.7	41.6
	x_{min}	30	99	93	20	86	49	92	76	13	91	36
	x_{max}	36	112	103	26	95	63	103	82	21	101	48
	SD	2.28	4.07	3.09	1.77	2.67	4.95	4.07	1.96	2.39	2.83	4.53
	CV	6.90	3.84	3.15	7.92	2.94	8.83	4.19	2.46	13.92	3.02	10.88
Mačkovac	$\overline{X}$	30.4	102.9	98	21.7	92	53.2	95.3	76.0	14.9	97.6	38.8
	x_{min}	27	98	92	19	89	49	83	72	14	89	33
	x_{max}	33	109	103	24	96	60	102	80	16	105	45
	SD	2.32	3.41	3.33	1.70	2.36	3.36	5.54	3.05	0.87	5.27	4.05
	CV	7.63	3.32	3.40	7.85	2.56	6.31	5.81	4.02	5.88	5.40	10.44
Total	$\overline{X}$	31.43	108.50	98.33	22.13	91.67	54.53	96.83	76.77	15.40	96.00	40.33
	x_{min}	27	98	92	19	86	49	83	70	12	89	33
	x_{max}	36	120	103	26	98	63	104	82	21	105	48
	SD	2.30	4.12	2.73	1.58	2.38	3.86	4.83	2.37	1.68	4.07	3.71
	CV	7.33	3.85	2.78	7.14	2.59	7.05	4.99	3.09	10.84	4.22	9.20
F		4.01*	9.92**	0.72^{ns}	1.04^{ns}	1.27^{ns}	1.38^{ns}	0.85^{ns}	10.18**	8.0**	2.37^{ns}	1.39^{ns}
Lsd 0.05		2.11 2.85	3.82 5.16	2.68 6.62	33.46 45.19	2.19 2.96	3.61 4.88	4.47 6.03	2.22 2.99	1.65 2.23	3.85 5.19	3.49 4.71
0.01												

Angle E9 had an average value of 22.13°, and in comparison to the previous angle had a slightly higher coefficient of variation (CV=7.14). The resulting differences in the mean values between different sites were not significant. In studies of Stojanović (2002), this angle had lower average value (19.50), lower extremes (18.4 and 20.59) and a lower CV (3.83).

Angles G18 (91.67), J10 (54.53) and J16 (96.83) had very similar values in all three locations from which the bees were sampled. Except J10 (7.05), all had low coefficients of variation, and analysis of variance showed that the differences between sites were not statistically significant. The results for the angles G18 and J10 are consistent with the values obtained in the research by Nedić et al. (2011), while the results for angle J16 (91.44) differ from those.

Angle K19 had a mean value of 76.77°, with a coefficient of variation of 3.09. The analysis of variance revealed that the location had a very significant impact on the values for this angle. Values for this angle in honey bees from site Majdevo (79.5°) were statistically significantly different from the values obtained in bees from the site Mačkovac (76°), and very significantly different from the site Makrešane (74.8°).

Angle L13 (15.4), after the angle B4, showed the largest variation between sites (CV=10.84). Particularly high coefficient of variation was found in honey bees from Makrešane (12.71°) and Majdevo (13.92°), while in Mačkovac the value for this angle was significantly lower (5.88). The analysis of variance showed that the location had a very significant effect on the expression of these traits. Values obtained in the sites Makrešane and Mačkovac did not differ significantly while the values obtained in Majdevo were very significantly different in relation to the others.

Angle N23 (96°) had a very small differences between sites and, therefore, had lower CV (4.22). Location did not significantly affect the value of this angle, and the differences between sites were not statistically significant. Angle O26 (40.33°) had a slightly higher CV

compared to the previous (9.2°), but a lower standard deviation. Analysis of variance showed that the differences between localities were not statistically significant. The values obtained for the angles N23 and O26 were for 3-40 degrees higher compared to the results obtained in research of Nedić et al. (2011).

Conclusion

Based on measurements of 11 angles on the front wing of honey bees from three different sites in the wider area of Kruševac in central Serbia, it can be concluded:

- The samples from the site Makrešane had the highest average values for angles B4, D7, E9, G18 and J16. Honey bees from the site Mačkovac had the highest average value for the angle N23. The highest average values for the angles A4, J10, K19, L13 and O26 were determined in honey bees from the site Majdevo.
- The highest variability for angles A4, D7, E9, J16, K19 and N23 was found in bees from the site Mačkovac. Honey bees from the site Majdevo showed the highest variability for the angles G18, J10, L 23 and O26, and bees from the site Makrešane showed the greatest variability for the angle B4.

The analysis of variance showed that the site has a significant impact on the size of the angle A4, and a very significant impact on the size of the angles B4, K19 and L13.

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SILAGE QUALITY OF INBRED LINES DERIVED FROM LOCAL MAIZE POPULATIONS

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Abstract

The present study aimed to investigate the silage quality, derived from whole plant biomass, of six experimental inbred lines derived from local maize populations. Chemical composition of the silage inoculants and control without the use of inoculants (content of free and total acids) and nutrient content (crude ash, crude fiber crude protein, crude fat, NFE, NDF, ADF), were investigated.

The results indicate that the silage of the whole plant of maize inbred lines can have a good quality.

Content of ash had numerically lower values in DM of silages with inoculant than maize silages without inoculant. Crude protein content was higher in LC4 (72,60% vithout inoculant; and 77.10% in treatment with inoculant). Line LC3 had the lowest content of crude fiber (299.7% without inoculant, and 288.8% with inoculant). The content of NFE was higher in the silages with inoculant ($P< 0.01$) than in silages without inoculant. The neutral detergent fiber (NDF) was significantly (inbred lines LC1, 2, LC3 and LC5) or numerically (inbred line LC6) lower in inoculated silages, than in silages without inoculant. The content of ADF were also significantly lower in the silages with inoculant compared to treatment with inoculant.

The amount of dry matter was highest in line LC6 (280,0 g kg^{-1} DM in variant without inoculant), and in line LC4 (301,3 g kg^{-1} DM in variant with inoculant). The pH of all silages tended to be under 3.55, which is considered to be acceptable for maize silages. Crude protein content in all maize silages were numerically higher in inoculated silages compared to silages without inoculant. The lowest content of lactic acid was detected in DM of inbred line LC4 (61.03 g kg^{-1} DM without inoculant, and 95.07 g kg^{-1} DM with inoculant). In our investigation, addition of inoculant slightly decreased lactic acid to acetic acid ratio, except in inbred line LC6 where addition of inoculant increased lactic acid to acetic acid ratio.

Maize silages treated with inoculants were more stable, because inoculant successfully stimulated the production of lactic and acetic acid which is antimycotic and enhanced aerobic stability.

Keywords: *inbred lines, genetic diversity, silage, nutrient content*

Introduction

A large number of maize hybrids with different genetic origin and purpose are present in agricultural Serbian market. Domestic hybrids are showed great competitiveness in such conditions.

Pollak et al. (2003), asserted that old maize varietes could be a significant source of quality features. In the process of obtaining and developing hybrids, comprehensive examination of starting material is very important (Bertoia et al., 2002).

In some rural areas of Serbia, we can still find old varieties of maize which, for many years of cultivation, became the native population of that area. In order to preserve genetic diversity, it is important to collect this material. During the period 1997-2000, such of this populations

were collected (in the willages of Stara Planina mountain) and begin the process of obtaining inbred lines. Extensive studies in the laboratory and in experimental plots were conducted (Milenkovic et al., 2012, 2014a, 2014b, 2015), and the results indicate the high quality of grain and biomass of this inbred lines.

Due to the high biomass production in just one growing season, maize is very widespread culture for silage production. In the area of Kruševac, production of maize for silage are represented to a greater extent, due to the favorable conditions of climate and soil. There is a large number of mini farms in this region (Milenkovic et al., 2015).

After the testing of suitability from silage, from whole plant of maize inbred lines originating from local populations (Milenkovic et al., 2015), the aim of this study was to investigate silage quality from the same material.

The ensiling process allows preservation of forage in long period. Silages are preserved through a fermentation process that produces acids to inhibit spoilage.

Using biological additives (inoculants) in silage is a good way to improve the qualitative characteristics of silage, due to the intensification of lactic acid fermentation in materials with different amounts of soluble sugars (Djordjevic et al, 2004).

Maize silage quality is determined by energy content and content of protein and minerals. Evaluating silage quality include chemical, biological and instrumental methods. In this research we use chemical methods to determine silage quality.

Material and methods

Inbred lines are planted in a growing season 2012, according to a randomized block design with two replications. At the stage of milk ripeness of grain, a representative sample of plant biomass from maize was taken. For the preparation of silage 10 plants of each genotype was taken. Whole plants are chopped (mixed and equable for the standardizing), then the sample was taken for silage.

Ensiling biomass was carried out in experimental containers holding 60 dm^3. For each genotype were used by 4 courts (two replications and two treatments: without inoculant-A_0 and with inoculant-A_1). In one treatment is added 1 g per 100 kg of green mass of inoculant Sill-All (Alltech). The inoculum was previously dissolved in distilled water and uniformly distributed in the powdered mass. In order to achieve uniform moisture both treatments, without inoculant is sprinkled with the same amount of distilled water.

The trial was set up according to the model a factorial experiment (with two variants: with and without inoculant), where the factor is genotype. The courts were opened after 90 days and quality parameters and chemical composition were tested (AOAC, 1984). In the lab, they made the following chemical analysis: Dry Matter (DM is percentage of the biomass without water); Crude Protein (CP) is the total amount of nitrogen (N percent multiplied by 6.25 equals CP); Crude Fat (CF), and Crude Ash.

We examined the content of the following nutrients in the silage: Neutral Detergent Fiber (NDF is the percent of cell wall material in a forage); Acid Detergent Diber (ADF is a highly indegestible percent of plant material); Lactic Acid is colorless, liquid organic acid resulting from fermentation; Acetic Acid is corosiv, colorless organic acid with pungent odor; Butyric Acid is viscous, foul-smelling, liquid carboxylic acid. In our material there was no content of butyric acid.

The results were statistically analyzed using the program Minitab. For comparison the middle of treatment was used Tukey test (Snedecor and Cochran, 1982).

Results and discussion

Crude protein content (CP), in all silages from maize, was numerically higher in inoculated silages compared to silages without inoculant (Table 1). Koc et al. (2008) found a decrease

(P< 0.01) in CP content in inoculated silages. Baytok et al. (2005) found that higher concentration of the same inoculant did not affect CP concentration in maize silage. Despite the crude protein, concentration of crude fiber decreased in silages with inoculant, except in DM of inbred line LC5, where content of crude fiber increased in silage with inoculant.
Content of ash had numerically lower values in DM of silages with inoculant than maize silages without inoculant. The highest content of crude fat was in silage with inoculant in DM of inbred line LC6 (40.03 g kg^{-1} DM) and the lowest content in silage without inoculant made from inbred line LC2 (21.77 g kg^{-1} DM). The content of NFE was also higher in the silages with inoculant (P< 0.01) than silages without inoculant (Table 1).
The neutral detergent fiber (NDF) was significantly (inbred lines LC1, LC2, LC3 and LC5) or numerically (inbred line LC6) lower in inoculated silages, than in silages without inoculant. In silage made from inbred line LC4 content of NDF was higher in treatment with inoculant (751.9 g kg^{-1} DM) than in treatment without inoculant (728.9 g kg^{-1} DM). High NDF may be the result of stress during the maize growing season, because 2012 was very dry, with high temperatures. The content of ADF were significantly lower in the silages with inoculant inclusion (silages from inbred lines LC1, LC3 and LC5) compared to those silages from inbred lines LC2, LC4 and LC6 where treatment with inoculant had higher content of ADF.

Table 1. Content of nutrients in maize silage g kg^{-1} DM

Inbred line		Ash	Crude proteins	Crude fiber	Crude fat	NFE	NDF	ADF
LC1	A_0	80,73 C	65,83 G	334,8 A	23,00 G	498,0 F	695,3 E	483,0 A
	A_1	78,03 D	70,67 E	305,1 F	26,20 F	519,4 D	643,4 I	422,8 B
LC2	A_0	87,03 A	71,03 DE	330,4 B	21,77 H	486,8 H	666,7 F	418,2 C
	A_1	82,80 B	74,50 B	325,5 C	27,50 E	494,8 G	660,8 G	421,1 BC
LC3	A_0	77,90 D	70,23 E	299,7 G	22,00GH	532,9 C	656,0 H	383,2 E
	A_1	69,77 E	72,00 CD	288,8 H	21,97GH	555,0 A	613,5 J	367,9 F
LC4	A_0	81,10 C	72,60 C	331,3 B	36,87 B	489,3 H	728,9 C	398,6 D
	A_1	77,33 D	77,10 A	313,8 D	31,53 CD	504,0 E	751,9 A	400,1 D
LC5	A_0	81,83 BC	68,13 F	327,0 C	30,43 D	495,4 FG	705,4 D	422,6 B
	A_1	78,20 D	70,17 E	331,3 B	22,97 G	501,7 E	696,9 E	420,5 BC
LC6	A_0	59,83 F	65,17 G	310,7 E	32,60 C	531,3 C	735,0 B	385,9 E
	A_1	54,73 G	70,93 DE	289,8 H	40,03 A	542,3 B	734,3 B	383,9 E

Tukey test; P=0.01

The fermentation characteristics of investigated maize silages are given in Table 2. Dry matter silages were higher in treatments with inoculant, with exception for inbred line LC1 where DM was lower in treatment with inoculant than in treatment without inoculant, and for inbred line LC6 where DM was equal in both treatments, with and without inoculant. Similar results

were presented by Jolč et al. (2010) and Koc et al. (2008) where the mixture of inoculants and enzyme were used in maize silages.
The pH values of silage with inoculant decreased, and all treated silages had lower pH than untreated silage. The pH of all investigated silages tended to be under 3.55, which is considered to be acceptable for maize silages (Kung and Shaver, 2001). The ammonia-nitrogen content was highest in silage from inbred line LC2 without inoculant, and the lowest in the same silage with inoculant.

Table 2. Chemical analysis of whole plant silage on the lactic-acid fermentation

Inbred line		Dry matter	pH	NH4-N	H2O-N	Acetic acid-free	Total acids	
							Acetic	Lactic
LC1	A_0	229,3 G	4,2 B	0,733 DE	6,100 C	40,83 F	49,40 E	85,80 F
	A_1	220,7 H	3,8 G	0,533 FG	6,400 B	70,83 A	74,90 A	120,53 A
LC2	A_0	238,3 F	4,1 CD	1,367 A	6,600 B	25,90 J	36,27 G	96,83 D
	A_1	248,7 E	3,9 F	0,433 G	6,000 C	45,77 D	50,70 D	105,80 B
LC3	A_0	240,7 F	4,0 DE	0,833 CD	5,600 D	29,80 H	33,07 H	101,47 C
	A_1	269,0 C	3,8 G	0,667 EF	6,400 B	32,67 G	48,83 E	100,77 C
LC4	A_0	298,3 A	4,4 A	0,833 CD	4,433 F	25,77 J	33,60 H	61,03 I
	A_1	301,3 A	4,0 DE	0,967 BC	4,100 G	56,87 B	60,90 B	95,07 E
LC5	A_0	261,0 D	3,9 E	0,967 BC	5,533 D	27,80 I	29,93 I	82,97 G
	A_1	279,3 B	3,8 FG	0,967 BC	5,067 E	50,93 C	57,70 C	107,30 B
LC6	A_0	280,0 B	4,1 BC	1,000 B	6,600 B	32,97 G	38,83 F	75,50 H
	A_1	280,0 B	3,9 F	1,067 B	7,567 A	43,60 E	50,97 D	106,03 B

Tukey test; P=0.01

Inoculant-treated silages were more stable, because inoculant successfully stimulated the production of lactic and acetic acid which is antimycotic and enhanced aerobic stability. The concentration of butyric acid was not detectable.
Lactic acid should be at least 65-70% of the total silage acids in well fermented silage (Shaver, 2003). Our results are in consistant with the results obtained by Shaver (2003), who indicated that lactic acid could have higher values. The lowest content of lactic acid was detected in DM of inbred line LC4 (61.03 g kg^{-1} DM without inoculant and 95.07 g kg^{-1} DM with inoculant). The ratio of lactic acid to acetic acid is a good indicator of the efficiency of the silage fermentation. Ideally, the ratio of lactic acid to acetic acid should not be less than 3:1, and a higher one is beter (Kung and Shaver, 2001). In our investigation, addition of inoculant slightly decreased lactic acid to acetic acid ratio, with exception for inbred line LC6 where addition of inoculant increased lactic acid to acetic acid ratio.

Conclusion

This study showed that ensiling maize maintains good silage quality. All the silages were well preserved as indicated by low pH, low ammonia and no butyric acid. The low pH, together with elevated levels of lactic acid showed that the herbage contained sufficient amounts of soluble carbohydrates to effectively preserve the silage.

Inoculant affect the intensification of lactic acid fermentation and increase the amount of lactic acid, reducing the pH, and complete absence of butyric acid.

The results of silage quality indicate that the investigated maize inbred lines can be used in the further process of getting a quality silage hybrids.

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CHARACTERISTICS OF RED CLOVER DRY MATTER FOR IMPROVING NITROGEN UTILIZATION IN RUMINANTS

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Abstract

Improving the efficiency of use of feed nitrogen (N) has become a central component of the ration formulation process primarily to reduce feed costs and also for the desire to be more environmentally friendly. New data are available that better describe the characterization of feed fractionation and these data along with changes in available models suggest that more protein has been available to lactating cattle than previously considered. Evaluation of the current data are suggesting that how we currently characterize feed protein fractions and their associated degradation and passage rates might cause us to over-feed protein. This study was conducted to assess effects of cultivar and cut on protein fraction content using a factorial experiment with a randomized complete block design. Samples of red clover *cv* K-32 (tetraploid cultivar) and *cv* K-39 (diploid cultivar) was taken at mid-bloom stage on May the 21st in the first cut, on June the 18th in the second cut and on July the 9th in the third cut, at the same stage of growth. Crude protein content increased from the first to the third cut in both investigated cultivars of red clover, but *cv* K-32 had higher content of crude protein than *cv* K-39 ($P< 0.05$). The neutral detergent insoluble protein was lower in dry matter of tetraploid cultivar of red clover, while, the acid detergent insoluble protein values were low in both cultivars, indicating that forages had not been subjected to heat damage. Cultivar differences in soluble protein, true protein and non-protein nitrogen were significant, and the highest content of insoluble protein and true protein were detected in the third cut. Our results confirm that there is significant variability in protein fractions and degradability among red clover cultivars. The foregoing discussion leads to consideration of ways that legume forages might be altered to improve the nutritional value of their proteins for productive ruminants.

Keywords: *protein fractions, nitrogen utilization, red clover*

Introduction

Red clover (*Trifolium pratense* L.) is commonly grown throughout the world. It is an important perennial forage legume because it is used as hay or as pasture in crop rotations (Fornham and George, 1993) and has high productivity and protein content (Murray et al., 2007). It is especially suitable for cattle breeding, because it has quality and nutritive hay (Acikgoz, 2001).

The main storage components accumulated by red clover are total protein, which can make up as much as 21% of the dry matter. Both di- and tetraploid forms of red clover are grown commercially. The two forms have different morphological traits and agricultural characteristics. Zuk-Golaszewska et al. (2006), who analysed yields of di- and tetraploid cultivars of red clover demonstrated that red clover tetraploid forms were superior in production of total protein yields.

At present, the quality-orientated breeding of clover focuses on improving the content and quality of proteins (the amino acid composition, sensitivity to proteolysis, polyphenol oxidase activity) delaying the lignification process as plants mature or increasing the share of carotens and decreasing the concentration of anti-nutritional compounds, e. g. isoflavones

(Lee et al., 2004). Under these economic and environmental constraints, improving the efficiency of N utilization and reducing N excreted are very important to maintain the sustainability of dairy farms, and nutrition models have become an effective farm management tool to accomplish these tasks (Lanzas et al., 2007). Milk production will be reduced when protein supplied by the diet is below energy allowable milk production, which is affected by protein degradation rates (Fox et al., 2004). Feed protein fractionation systems have been integrated into nutrition models to account for differences in protein availability and utilization. The *in situ* techniques and schemes based on solubility in buffers and detergent solutions have been adapted by the NRC (2001) and the Cornell Net Carbohydrate and Protein System (CNCPS, Fox et al., 2004) to measure protein fractions in feeds.

The objective of this study was to investigate the effect of variety of red clover grown in Serbia in different cuts on crude protein content and to determine the component of CNCPS model containing NPN (Non protein Nitrogen), SolP (Soluble Protein), NDICP (neutral Detergent Insoluble Crude Protein), ADICP (Acid Detergent Insoluble Crude Protein), TP (True Protein) and IP (Insoluble Protein).

Materials and methods

This experiment was carried out in the experimental field of Institute for forage crops in Kruševac (43^{o}34`58``N, 21$^o$19`35``E) from May to July in 2012. The study area was situated at altitude of 166 m above sea level in Central Serbia. The mean annual temperature and the total precipitation for the region are 12.6º C and 653.2 mm, respectively. Climatic dates of research peroid and long terms averages are reported in Table 1.

Table 1. Climatic dates of the research location in 2009, 2010, 2011 and 2012 years and long-term average (1968-2008)

Year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Total/ Mean
Mean Temperature (º C) (Monthly)													
1968-2008	-0.1	2.1	6.8	11.6	16.4	19.9	21.6	21.3	16.8	11.5	6.4	1.4	11.3
2009	1.2	3.1	7.7	14.0	18.3	20.7	22.8	22.6	18.7	12.2	9.0	4.0	12.8
2010	1.4	2.8	7.5	12.6	16.8	20.4	22.2	22.7	17.6	10.2	12.1	3.3	12.5
2011	-0.1	0.4	6.7	12.2	15.6	20.7	22.3	22.7	21.2	10.9	3.7	4.0	11.7
2012	0.6	-4.1	8.3	12.9	16.2	22.5	25.4	24.1	20.5	14.6	9.6	0.9	12.6
Total Precipitation (mm) (Monthly)													
1968-2008	38	36	43	57	70	76	60	47	51	50	55	53	636
2009	48.6	63.9	60	20.2	37.5	106.8	26.8	57.7	36.6	120.6	58.7	109.4	746.8
2010	35.1	109.6	46.4	79.7	69	114.4	57.5	34.6	15.6	83.8	23.9	83.9	753.5
2011	29	44	48	19	47	66	104	0	8	33	0	42	440
2012	118	62.1	20	69.1	150	29.3	33.3	0.0	15.2	55.4	12.2	88.6	653.2

The experiment was designed as a factorial trial, by randomized block system in three replicates. Samples of red clover, *cv* K 32 – tetraploid (4n) *cv* and K 39 – diploid (2n) *cv* were taken at mid-bloom stage on May the 21st in the first cut, on June the 18th in the second cut and on July the 9th in the third cut, at the same stage of growth. Dry matter was determined by drying out samples at 65º C and grinding and sieving them to 1 mm particle size. The samples were dried in an oven of 105º C for 6 h for dry matter determination.

The CP (Crude Protein) of the samples was determined using Kjeldahl method. The CP in the forage samples were partitioned as proposed by Cornell Net Carbohydrate and Protein System, CNCPS (Licitra et al., 1996). Soluble CP (SolP) was determined using the borate-phosphate buffer. Non-protein N (NPN) was calculated as the difference between the total CP and True protein (TP) precipitated with threechloracetic acid. Neutral Detergent

Insoluble Crude Protein (NDICP) was determined after NDF determination and Acid Detergent Insoluble Crude Protein (ADICP) was determined after ADF determination.
Data were processed by the analysis of variance in a randomized block design. Effects were considered different based on significant (P< 0.05) F ratio.

Results and discussion

In 2012, precipitation and temperatures during active forage growth were near long terms norms in April and May, while June and July was hot and dry. Except for a dry June and July, precipitation in 2012 greatly exceed long-term norms from the beginning of the year (except March), whereas temperatures were relatively high in June and July, but near normal from March to May (Table 1).

Following the mean values of crude protein content for cultivars and cuts, tetraploid *cv* featured higher CP content (P< 0.05) and the highest value of CP was in the third cut (Table 2). Previous work with three-cut harvest systems indicated bud-stage red clover from spring growth was lower in CP than subsequent summer cuttings taken at comparable maturity (Wiersma et al., 1998). Therefore, earlier cutting of spring growth may improve CP concentrations and their uniformity across harvest.

Table 2. Content of Crude Protein and protein fractions in red clover, g kg^{-1} DM

		b_1	b_2	b_3	$\overline{X}_A$
CP	a_1	**165.0$^{a, C}$**	**175.0$^{ns, B}$**	**195.0$^{ns, A}$**	178.3^{a}
	a_2	**150.6$^{b, C}$**	**171.9$^{ns, B}$**	**191.9$^{ns, A}$**	171.4^{b}
	$\overline{X}_B$	157.8^{c}	173.4^{b}	193.4^{a}	
NDICP	a_1	**26.9$^{b, B}$**	**23.1$^{b, C}$**	**37.2$^{ns, A}$**	29.0^{b}
	a_2	**47.5$^{a, A}$**	**26.4$^{a, C}$**	**35.6$^{ns, B}$**	36.5^{a}
	$\overline{X}_B$	37.2^{a}	24.8^{b}	36.4^{a}	
ADICP	a_1	**15.0$^{ns, C}$**	**27.5$^{a, A}$**	**19.6$^{ns, B}$**	20.7^{ns}
	a_2	**17.2$^{ns, B}$**	**20.2$^{b, AB}$**	**21.3$^{ns, A}$**	19.6^{ns}
	$\overline{X}_B$	16.1^{c}	23.9^{a}	20.4^{b}	
IP	a_1	**100.4$^{b, C}$**	**108.5$^{a, B}$**	**121.3$^{ns, A}$**	110.0^{ns}
	a_2	**107.5$^{a, B}$**	**97.5$^{b, C}$**	**123.9$^{ns, A}$**	109.6^{ns}
	$\overline{X}_B$	103.9^{b}	103.0^{b}	122.6^{a}	
SolP	a_1	**64.6$^{a, NS}$**	**66.4$^{b, NS}$**	**71.0$^{ns, NS}$**	67.3^{a}
	a_2	**43.1$^{b, B}$**	**74.4$^{a, A}$**	**69.8$^{ns, A}$**	62.4^{b}
	$\overline{X}_B$	53.8^{b}	70.4^{a}	70.4^{a}	
TP	a_1	**104.6$^{a, B}$**	**102.7$^{b, B}$**	**138.1$^{b, A}$**	115.1^{b}
	a_2	**96.6$^{b, C}$**	**124.4$^{a, B}$**	**150.6$^{a, A}$**	123.9^{a}
	$\overline{X}_B$	100.6^{c}	113.5^{b}	144.4^{a}	
NPN	a_1	**60.4$^{a, B}$**	**72.3$^{a, A}$**	**56.9$^{a, B}$**	63.2^{a}
	a_2	**53.9$^{b, A}$**	**47.5$^{b, B}$**	**43.1$^{b, B}$**	48.2^{b}
	$\overline{X}_B$	57.2^{a}	59.9^{a}	50.0^{b}	

Different letters denote significantly different means (P< 0.05)

Comparing different red clover cultivars means, tetraploid cultivar was higher (P< 0.05) in SolP and NPN, but lower (P< 0.05) in NDICP and TP, indicating large and potential differences in rumen CP degradation characteristics between tetraploid and diploid cultivars of red clover. The cut was an important source of variability for all investigated protein

fractions. The results indicate that red clover had the highest content of NDICP, IP and NPN in the first cut. The contents of IP were similar in the first and the third cut of red clover, but the lowest value ($P< 0.05$) was observed in the second cut. Therefore, content of ADICP and SolP were the highest in the second cut, and TP content was the highest in the third cut (Table 2).

Soluble crude protein is a simplastic concept that evolved out of the observation that most soluble nitrogen components were rapidly degraded in the rumen and therefore, reduced protein that could be passed to the lower tract. Protein solubility was significantly different among investigated cultivars of red clover ($P< 0.05$). Somewhat higher values were obtained in the second and third cut (Table 2). There was no statistical difference between the cultivars of red clover obtained in the third cut. In models designed to assess utilization of dietary protein by ruminants, it is assumed that most of the soluble protein is completely degraded in the rumen and varying proportions of the insoluble fractions escape ruminal degradation depending on the interactive effects of digestion and passage (Sniffen et al., 1992). Because various protein fractions differ in rate and extent of ruminal degradation, the proportions of these different protein fractions in feedstuffs are believed to influence the amount of ruminally degraded and escape protein consumed by animals (Elizalde et al., 1999).

The differences in the content of protein fractions were relatively small, but diploid cultivar of red clover had much more NDICP ($P< 0.05$) in the first cut, and much lower SolP ($P< 0.05$) than tetraploid cultivar of red clover. The highest content nof NDICP was found in the first cut (37.2 g kg^{-1} DM or 23.6% of CP). Comparing the two varieties of red clover there were no differences ($P> 0.05$) in ADICP in the first and the third cut and in IP in the third cut. Diploid cultivar of red clover, K 39, was higher in TP, but lower in NPN ($P< 0.05$) than tetraploid cultivar of rd clover–K 32, indicating potential differences in rumen CP degradation characteristics between investigated red clover varieties. The weather conditions during the growth of plants and the form of red clover differentiated the concentration of protein fractions in DM of red clover. Harvest schedule had cut specific effects on all buffer-soluble CP fractions and on most detergent fractions. Grabber (2009) indicated that the early harvest schedule increased the proportion of readily degraded buffer-soluble CP and reduced the proportion of slowly degraded NDICP in the first cutting of red clover.

The nitrogen associated with NDF is normally cell wall-bound protein which also includes the indigestible nitrogen found in the acid detergent residue. The protein insoluble in the neutral detergent solution, but soluble in acid detergent solution is digestible, but slowly degradable. Generally, the cell wall-associated protein is extension covalently linked to hemicellulosic carbohydrate (glycoproteins) that are involved in cross linking carbohydrate chains in plant cell walls (Licitra et al., 1996).

The CNCPS model was only sensitive to NDICP measurements for feeds that contain high proportions of protein as NDICP (Lanzas et al., 2007). Reported values for NDICP degradation rates might be similar or slightly greater than NDF degradation rates (Lanzas et al., 2008). This fact has led to the question of the appropriateness of using the N isolated in detergent solution as an indicator of the slowly protein degradation factor. When protein degradation is very rapid, ruminal microbes can not utilize all of the amino acids and ammonia released and more protein will be degraded than is synthesized. Protein entering the rumen has at least three fates: it is degraded to ammonia and is used for bacterial protein synthesis, leaves the rumen as ammonia and converted to urea in the liver, or escapes microbial action becomes metabolizable protein directly.

The two most important considerations of feed CP chemistry are: the proportional concentrations of NPN and the true protein and the physical and chemical characteristics of the proteins that comprise the true protein fraction of the feedstuff (NRC, 2001). Differences in 3-dimensional structure and chemical bonding (i. e., cross-links) that occur both within and

between protein molecules and between proteins and carbohydrates are functions of source as well as processing. These aspects of structure affect microbial access to the proteins, which apparently is the most important factor affecting the rate and extent of degradation of proteins in the rumen (NRC, 2001).

Conclusions

Overall, the present study gives a deeper insight into CP fractions in the spring growth and summer regrowth period of different varieties of red clover. The CP fractions seem to provide common parameters for grassland scientists, who focus on plant breeding targeting the nutritive value linked to physiological processes in plants, and for animal nutritionists, who wish to improve protein quality in forage-based diets using CP fractions at specified harvest dates. Although, from the point of view of ruminant nutrition, red clover showed advantages for the NPN and ADICP fractions of CP. In general, the chemical CP fractionation provides, in addition to the classical characteristics such as energy or fibre content, further information to achieve a better evaluation of the quality of forage legume species. Moreover, the present study provides valuable data for the modelling of the CP fractions, which should be the aim of continuing investigations.

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VARIABILITY OF MILK UREA ON VOJVODINA'S DAIRY FARMS

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Abstract

Determination of milk urea (MU) is a relatively new test developed to assess dietary needs of lactating dairy cows. Concentrations of MU are variable from herd to herd and between cows in the same herd. Higher values of MU indicate an imbalance of protein and energy, but MU concentration is influenced by a whole range of factors too (feeding, breed, stage and number of lactations, body weight, daily production and chemical composition of milk, somatic cell count, season and milking).

The aim of this study was to determine MU concentration in cow milk from dairy farms in Vojvodina.

The determination was based on representative samples from four farms in Vojvodina, handled according to Dairy Herd Improvement (DHI), during for the period of one year. A total of 17774 collected samples, of Holstein cows, were shown milk urea content from 7.0 to 82 mg/dl, with the next average value of the farms (F): F1 23.84mg/dl, F2 29.47 mg/dl, F3 21.79 mg/dl and F4 20.45 mg/dl. Statistical analysis showed significant differences between farms on MU concentration.

Coefficient of variation for MU was higher (32.68 – 43.58%) compared with other milk production traits: fat percentage (17.69 – 24.97%) and protein percentage (12.3-13.93%).

In this research a negative correlation coefficient was determined between protein content and urea concentration in milk, on farms F2, F3 and F4, while only on farm F1 a positive correlation coefficient was determined. Variations of urea content were observed, affected by seasonal change.

Keywords: *dairy farm, milk urea, protein, season*

Introduction

Testing for milk urea (MU) is a relatively new test developed to assess dietary needs of lactating dairy cows. Urea is considered a normal component of milk and is part of the non-protein nitrogen (NPN) found in milk (Ferguson, 1999). Milk urea nitrogen (MUN) is primarily derived from blood urea nitrogen (BUN) because urea equilibrates with water in the body. This equilibrium explains the high correlation between MUN and BUN concentrations (Sosa et al., 2010). BUN derives from the liver detoxification of ammonia diffused from the rumen in the liver (DePeters and Ferguson, 1992). The normal/target values of MUN are considered to be within the range from 10 to 15 mg/dl (Carlsson and Peherson, 1994; Moore and Varga, 1996). High values of MU indicate an imbalance of protein and energy (Wood et al, 2003). The benefits of using MU as a monitoring also help to optimize the efficiency of dietary protein utilization which include improved efficiency and reduce cost of production and nitrogen excretion into the environment (Kohn et al., 2005, Stoop et al., 2007). Animal husbandry has been identified as a major contributor of N pollution to water resources. MUN is highly correlated with urinary N exertion (Jonker et al., 1998; Kauffman and St-Pierre, 2001) and is good indicator of NH_3 emission from dairy manure (Burgos et al., 2007).

MU is varied from herd-to-herd and between cows in the same herd (Arunvipas et al, 2003). Concentrations of MUN may also differ even if cows consuming the same diet, due to genetic differences in the ability to metabolize protein (Wood et al, 2003). Aguilar et al. (2012)

considered that genetic diversity also exists among cows. MU might be used as a selection tool, and therefore, information on factors affecting it is needed (Stoop et al, 2007).
The aim of this study was to determine MU concentration in cow milk from dairy farms in Vojvodina.

Materials and methods

Raw milk samples were obtained from four farms in Vojvodina (Bačka), by the ICAR (2012) AT_4 method. All samples in this study were taken during milking time, once a day. Herd sizes ranged from 300 to 700 cows. Cows on Farms 1, 2 and 3 were housed in tie stall barns, and on Farm 4 were housed in free stall barn. The averages of 305 days milk production were on F1 8,586 kg/cow, F2 7,359 kg/cow, F3 8,045 kg/cow and F4 8,045 kg/cow. It was analyzed 17774 milk samples in total. The majority of the cows belong to Holstein breed (black and red). In generally, the feeding of dairy cows was using total mix ration with the most common management practice on the dairy farms in Vojvodina. Samples were collected in 40 ml bottles, containing potassium dichromate ($K_2Cr_2O_7$) as preservative and kept on temperature 1-4°C. Individual test-day milk samples were analyzed in the Laboratory of milk quality, at the Faculty of Agriculture in Novi Sad. Analysis of milk samples were performed on Foss's instrument MilkoScan^{TM}FT. To convert MU (milk urea) to MUN (milk urea nitrogen), the following conversion formula can be used:

MU (mg/dl) x 0.467 = MUN (mg/dl), Oudah (2009).

Extreme values of particular parameters were not analyzed in the following cases:
Milk urea content less than 7 and more than 90 mg/dl was excluded.
Milk fat percentage less than 2 and more than 6 was excluded.
Milk protein percentage less than 2 and more than 5 was excluded.
Milk urea was grouped into seven categories by increments of 5 mg/dl, started with those less than 10 mg/dl and finishing with concentration greater than 35 mg/dl.
Statistical data analysis was carried out using program STATISTICA 12. Statistical analyses included means values, standard deviation for individual parameters, coefficient of variation and correlation coefficient between protein and MU content in milk.
Using method of analysis of variance it was tested the difference in milk fat, protein and urea content between farms. Using the t –test the average milk fat, protein and urea content values between farms were compared.

Results and discussion

The mean value of MU for all the farms, during the one year, was 23.06 mg/dl. Results for MU for four farms were F1 23.83 mg/dl (MUN 11.13mg/dl); F2 29.47 mg/dl (MUN 13.76mg/dl); F3 21.79 mg/dl (MUN 10.17mg/dl) and F4 20.45 mg/dl (MUN 9.55mg/dl) (Table 1).
Our results are similar to the values for MU obtained by Hof et al. (1997), Kauffman and St-Pierre (2001), Bastin et al. (2009) and Bandelja et al. (2011) for Holstein breed.
The higher values of MUN reported Broderic and Clayton (1997), Jonker et al. (1998), Johnson and Young (2003), Wood et al. (2003) and Oudah (2009).
Coefficient of variation for MU was high (32.68 – 43.58%) compared with other milk production traits: fat percentage (17.69 – 24.97%) and protein percentage (12.30-13.93%). Bastin et al. (2009) and Bandelja et al. (2011) also found a high coefficient of variations for MU (42% and 36.16%). Oudah (2009) found les CV for MUN (31.5%).

Table1. Farm results for milk fat, protein and milk urea content

Farm	N	%						mg/dl			
		Milk fat			Protein			MU			MUN
		Average	SD	CV	Average	SD	CV	Average	SD	CV	Average
F1	3097	3.5	0.91	2497	3.24	0,45	13,93	23,83	10,38	43,58	11,13
F2	3215	4.19	0.88	20.99	3.20	0.41	12.91	29.47	9.63	32.68	13.76
F3	5034	4.08	0.72	17.69	3.24	0.39	12.30	21.79	7.57	34.76	10.17
F4	6428	3.75	0.79	21.09	3.31	0.41	12.42	20.45	7.51	36.75	9.55
Total	**17774**	**3.91**	**0.84**	**21.47**	**3.26**	**0.42**	**12.82**	**23.06**	**9.10**	**39.46**	**10.77**

*N = number of milk samples

Wood et al. (2003) indicated that concentrations of MUN may also differ if cows are consuming the same diet. Aguilar et al. (2012) pointed on the significant effect of cow in their research and field trials is clear evidence of phenotypic differences in MUN concentrations among cows that are not explained by N intake, milk yield, body weight, or other production – related factors.

The results of F-test showed significant differences between the farms concerning milk fat, protein and milk urea content. Using t-test it was found statistically significant differences between averages of milk fat, protein and milk urea content on four farms ($p<0,01$ and $p<0,05$). Only the difference between the mean protein content between farms F1 and F3 was not statistically significant, Table 2.

Table 2. The values of t test between farms

Farms	F2			F3			F4		
	%		mg/dl	%		(mg/dl	%		mg/dl
	Milk fat	Protein	MU	Milk fat	Protein	MU	Milk fat	Protein	MU
F1	24.35**	3.27**	22.0**	22.98**	0.47	10.22**	5.61**	7.87**	18.06**
F2	-	-	-	6.64**	3.34**	40.35**	25.18**	12.21**	50.44**
F3	-	-	-	-	-	-	22.20**	9.94**	9.42**

$t_{0,05}$=1,96, $t_{0,01}$=2,58

On Figure 1 it can be seen that at the farms F3 and F4 with the highest percentage of milk samples had MU content in the range from 15 to 30 mg / dl (7.01 MUN – 14.01mg / dl), which is considered to be the normal physiological values of milk urea in cow's milk (Abdoul et al, 2008).

At farms F1 and F2, that percentage are was slightly lower. At farm F2 with increasing MU content the percentage of milk samples increasing too.

As can be seen in Figure 2, milk urea was higher during the spring and summer months. For all farms the highest mean value is was in May (32.54 mg/dl) and the lowest in October (18.14 mg/dl). Similar results have been reported by other authors (Carlsson et al., 1995; Godden et al., 2001, Abdouli et al. (2008), Čobanović and Perišić, 2014). Rajala-Schultz and Saville (2003) were observed that in the low producing herds MUN concentrations were the highest during summer and significantly lower in winter, spring and fall. In the high producing herds, the differences among seasons were much smaller, but MUN concentrations were lowest during summer. Hojman et al. (2004) reported higher concentration MUN in the spring and the highest in the beginning of the summer, in June (18.1mg/dl). Bastin et al. (2009) found the highest MU during summer months, especially in August, and the lowest during the month of February. This fluctuation could reflect the grazing period and the access of cows to fresh pasture, which typically contains highly degradable protein and has a high protein-to-energy ratio (Soriano et al., 2001).

In this research a negative coefficient of correlation was determined between MU and protein content, on farms F2, F3 and F4. Only on farm F1 a positive correlation coefficient was determined. In Figure 3, the protein content, for different MU (mg/dl) groups is displayed.
In majority of the researches a negative correlation coefficient between protein content and urea concentration in milk was determined (Godden et al (2001), Johnson and Young (2003), Rajala-Schultz and Saville (2003), Hojman et al (2004), Abdouli et al (2008).
Abdouli et al. (2008) suggested that when herd - level mean milk protein was 3.0 to 3.2% and MUN concentration was 12 to 16 mg/dl, protein degradability fractions and net energy were most likely balanced.
Oudah (2009) found that the lowest MU (<15 mg/dl) had the lowest protein percentage (3.38%), and meanwhile the highest MU category (>55 mg/dl) had the highest protein percentage (3.46%).

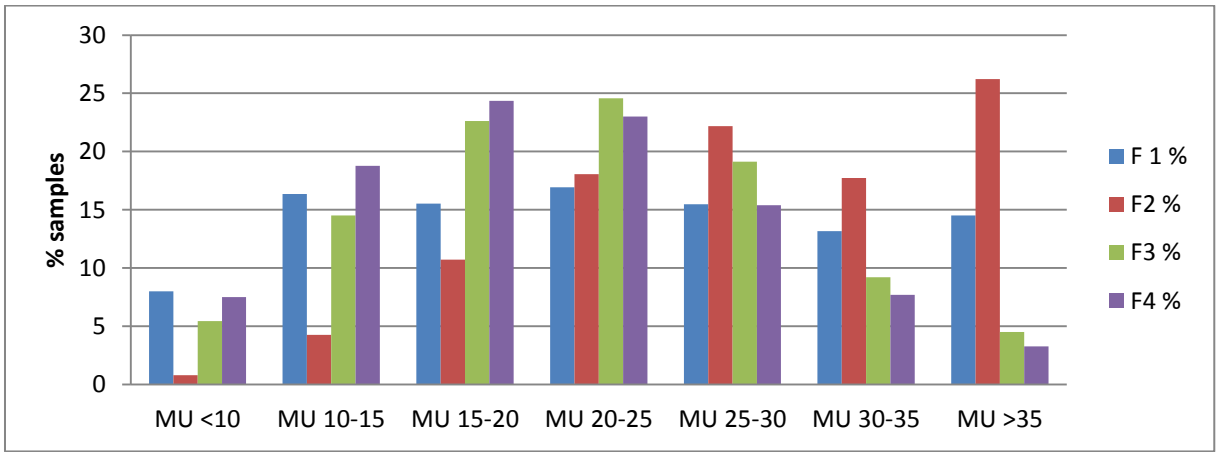

Figure 1. The distribution of milk samples per MU (mg/dl) groups

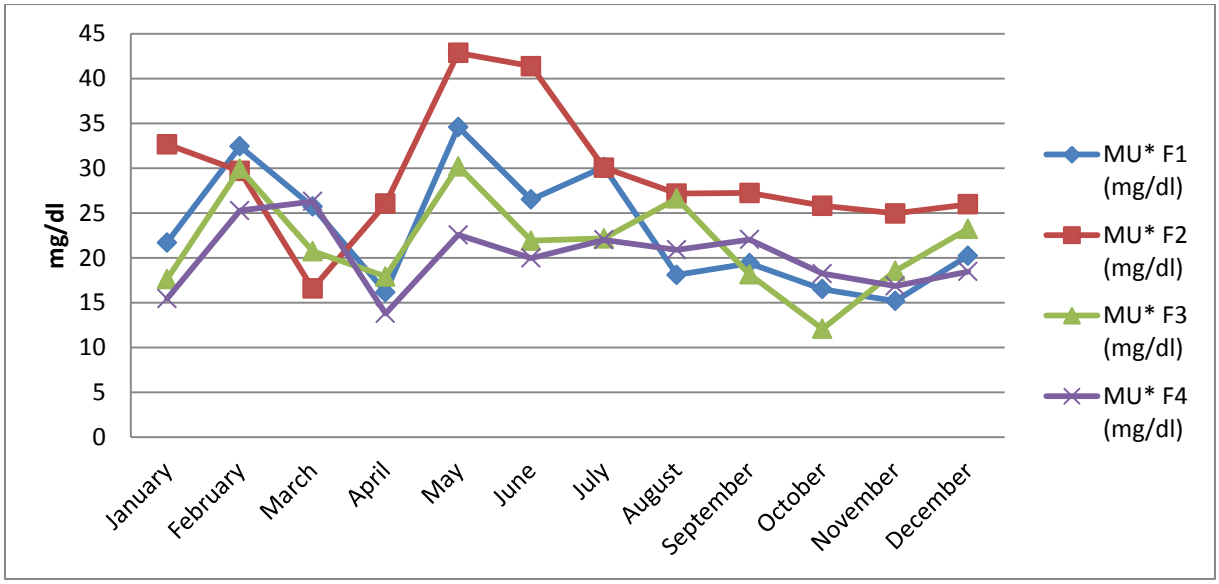

Figure 2. Content of MU (mg/dl) for farms by month of recording

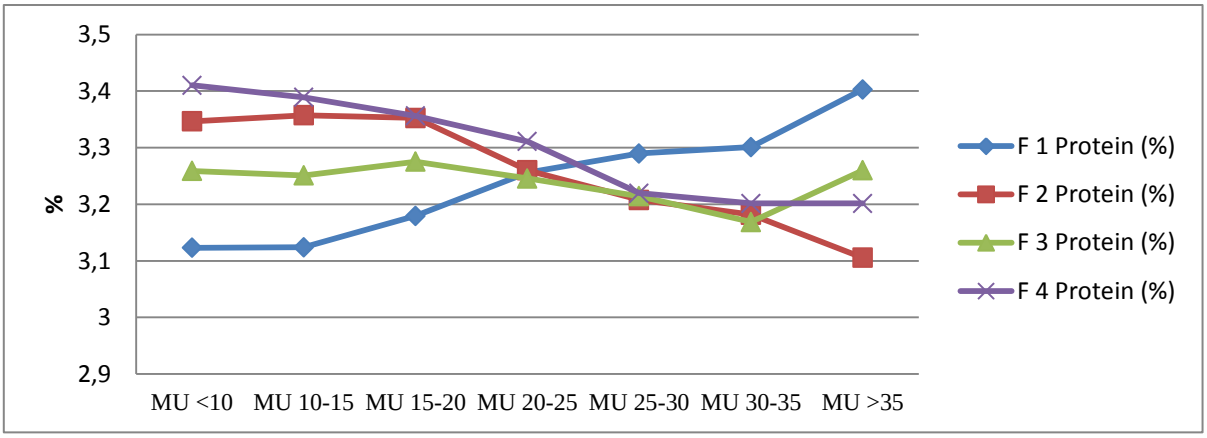

Figure 3. Protein content by MU (mg/dl) groups

Conclusion

The mean value of MU for all the farms, during the period of one year, was 23.06 mg/dl.
MU values for farms were 23.83 mg/dl (MUN 11.13mg/dl); 29.47 mg/dl (MUN 13.77mg/dl); 21.79 mg/dl (MUN 10.18mg/dl) and 20.45 mg/dl (MUN 9.55mg/dl) respectively.
Management at farms plays a great role as a source of variability in MU levels.
Compared with other milk production traits, fat percentage (17.69 – 24.97%) and protein percentage (12.3-13.93%), coefficient of variation for MU was higher (32.68 – 43.58%).
In this research a negative correlation coefficient between protein content and urea concentration in milk was determined, on farms F2, F3 and F4. Only on farm F1 was determined positive correlation coefficient. MU content varied according to season.
Using MU to monitor and adjust ration energy-protein balance might provide an opportunity to reduce feed costs and to improve profitability of the herd.

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Review paper
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IMPLEMENTATION OF THE „BREEDING PROGRAM” IN PIG PRODUCTION IN VOJVODINA PROVINCE (SERBIA)

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Abstract

The „Breeding program“(B.p.) is a set of breeding and selection methods and procedures which provide genetic improvement of pigs in Vojvodina province (Serbia). The application of appropriate breeding and selection methods according to the set goals represents the basis for pig production, while it maintains or improves vitality, increases efficiency, improves product quality and preserves genetic diversity.

The legal basis for the B.p is given in the Act of Livestock from 2009. First B.p came in 2010, and was enforcing by the end of 2014.The new B.p. which is a continuation of the previous one, with more strict evaluation criteria when selecting animals for breeding, was adopted in 2014 and entered into force in early 2015.

One of the selection methods which is prescribed by the B.p. is the selection and evaluation of the animals on the basis of data on individual abilities, or performance test. The aim of the performance test is to be used for breeding of superior animals in terms of their own phenotypes for a particular trait, where examined animals are the candidates for further selection.

Main breeding organization collects, processes and analyzes data related to recorded population at the territory of Vojvodina. Data were collected from primary and regional breeding organizations, through quarterly reporting documentation.

Based on the data of the main breeding organizations for the period from 2010 to 2014, the number of gilts in performance test increased by 24.54%, while the number of boars in performance test was reduced by 23.72%.Meat yield was increased to 56.35% in boars, and 57.9% in gilts, while the depth of MLD increased to 76.69mm and 77.6mm in boars and gilts respectively.

Application of B.p.in the previous period and the increase of breeding goals for the upcoming period will give a positive effect on important production traits that directly affect the cost of pig production in Vojvodina affecting that production will be more economical and cost effective. According to the mentioned above, it is concluded that the implementation of B. p. provides positive effects on pig production in Vojvodina.

Keywords: *Breeding programs, gilts, boars, pig, Vojvodina province*

Introduction

The implementation of breeding programs in animal husbandry is the primary activity of the Centre for the breeding of domestic animals at the Department of Animal Science, at the Faculty of Agriculture in Novi Sad. One of the most important segments of the breeding value is the genetic improvement - selection of domestic animals. Work on the selection of animals in Vojvodina, institutionally started by establishing the Regional Institute of Animal Science in 1950. Over the years, the structure of organizational functioning was repeatedly changed.

When the Law on Animal Husbandry entered into force (“Sl. glasnik” RS41/09), Department of Animal Science, at the Faculty of Agriculture, University of Novi Sad in 2010 received the "Decision on registration breeding organization of the Ministry of Agriculture, Forestry and

Water Management" as the Main breeding organizations in Vojvodina and in the same year it started the implementation of a major breeding program for pig farming. (Trivunovic et al., 2014).

Subjects in the implementation of Main breeding programs of pig farming in Vojvodina are: Farmers, Basics breeding organizations, Regional breeding organizations, Main breeding organization, Organizations with special authorization, Provincial Secretariat for Agriculture, Water Management and Forestry, Ministry of Agriculture and Environmental Protection.

The Main breeding program for pig farming in Vojvodina deals with a various number of different but also interrelated parts for the further development of pig production such as: the state of the pig, the conservation of genetic resources, animal welfare, genetic improvement (breeding), selection and testing of breeding animals, assessment of conformation breeding pigs and fattening pigs as well as issues related to the modernization of pig production in order to comply with European Union standards. The Main breeding goals are: increasing productivity of farm animals, changing and improving the racial composition and preventing decreasing in number of animals (Main breeding program, 2014).

Breeding programs can be considered as an investment in the context of long-term development programs that should be implemented in accordance with the national strategy for agricultural development while respecting existing limitations (finance, etc.). Breeding programs should be simple, direct and clear, unlike some other breeding programs for which program measures would not be guaranteed in time and experience (Kosgei et al., 2011). Above all is the implementation of the components of the breeding program, where it is necessary to monitor program results.

Programs for improving production characteristics which are applied today in pig breeding mainly consist of selecting the best breeding race, recommending of appropriate combination of race with the crossing and continuous improvement of production characteristics of pure breeds and the use of breeding animals which have a high breeding value. There is no doubt that there is a difference in breeding programs between certain countries and manufacturers (Merckx and De Vries, 2002).

Today the breeding program is based on the inter section of paternal and maternal lines and race, as well as performance testing and application of BLUP animal model, using PEST and PIGBLU software packages. For more precise estimation of the breeding values for the tested animals, it is necessary to use those models to include different factors affecting the variation characteristics (Mijatovicet et al., 2006; Trivunović, 1996). In Vojvodina province (Serbia), as a basic method to improve production performance is to use pig crossing. To produce commercial F1gilts, Landrace and Large White are used, while for the production of three-breed crossbred, F1 commercial gilts are intersect with terminal boars race (Trivunovic et al., 2014).

The breeding goal in swine production is focused on the repair of all economically important traits (life gain, back fat thickness, depth of MLD) so that total production could be bigger and more profitable (Radovic, 2011).

The most important goals of the pig breeding program in Vojvodina are the production of 21 fattening pigs per hybrid sow with 80% of yields of frozen carcass and 55-58% of meat in carcass, 880-928 kg of quality muscle tissue per year per sow, the necessary amount of food per piglet born alive of 60 kg, number of farrowing 2, 3 per sow per year, the number of piglets born alive per litter > 10.5, the total number of piglets per sow per year 24, the number of raised piglets per sow 22 (Main breeding program in Vojvodina province, 2014).

Materials and methods

The Main breeding organizations collect process and analyze data relating to the population of pigs in the territory of Vojvodina. Data are collected from Basic breeding organizations, through the Regional breeding organization by submitting quarterly reporting documentation. One of the selection methods prescribed by breeding programs is the selection and evaluation of the animals on the basis of data on individual abilities, i.e. performance test. Performance test represents a selection measure, performed by the Main organization of the breeding farms with an apparatus for ultrasonic measurement "Krautkramer", where the tested pigs are the candidates for further selection gilts and boars of pure race, as well as the F1 generation that are prescribed by Main breeding program. The very purpose of performance testing is to improve the performances of herd by increasing the frequency of desired genes, selecting the best rated animals, based on the results of performance test. The results of the Performance tests are processed in the General breeding organization using the software packages scheduled for making the selection index. The results of the test are sent to farms and on its basis the animals with positive results continue to be engaged in breeding, as the nucleus of the herd.

Results and discussion

Trivunović et al. (2014) recorded a decrease in number of tested boars, where it was observed a constant decrease which was particularly visible in 2011. (compared to 2005 by 43.6%). In 2013, this trend continued to declines lightly compared to the previous year. During the 2014, in the Autonomous Province of Vojvodina performance test was conducted on 987 boar which is an increase of tested boars for 12.92%, more than in 2013.

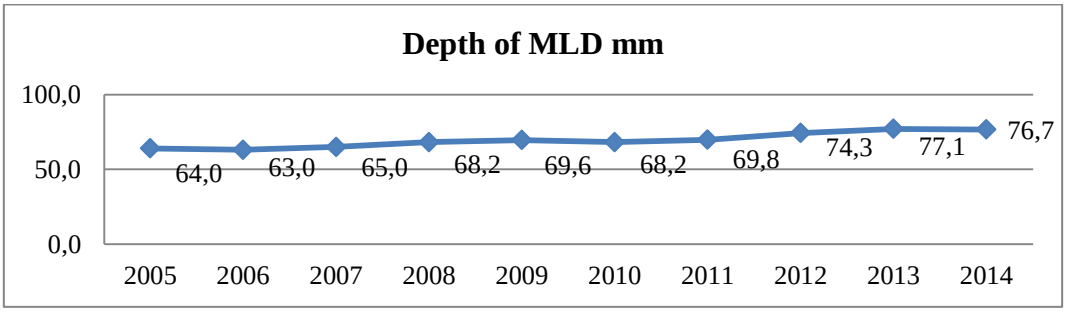

Chart 1. Depth of MLD in boars in the test from 2005-2014.

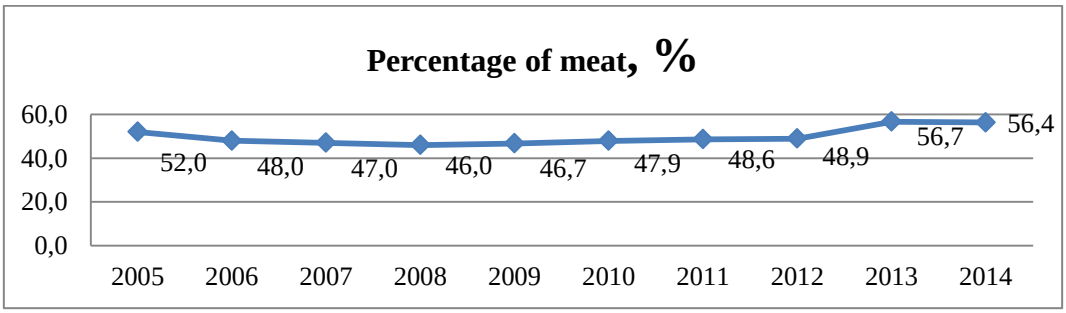

Chart 2. Percentage of meat in boars in the test from 2005-2014.

It appears from the chart (1 and 2) when it comes to the depth of MLD in boars, there had been a constant increase from 2006, while a slight drop was recorded in 2014. (76.7mm), an increase of MLD was recorded by Radovic (2010) for the period 2005-2009 and the increase was from 64 mm to 69.6 mm. When it comes to the percent of meat in boars, it was observed the increase of conformation of the meat in 2009 (46.70%), while the average leanness of

meat in 2014 was 56.4% (Chart 2). Radovic (2010) found that the average leanness of the meat in 2009 amounted to 46.7%. The trend of increasing has continued which leads to a more strict system of more stringent selection. Due to the high degree of heritability, genetic improvement can be achieved by using the performance test results. Based on the data of the test results (weight gain, feed conversion, back fat thickness, % of meat in carcasses, number of tits, constitution, size and number of litter from which originates),the best boars should be selected (Yoo and Lee, 2011).

What constitutes a significant improvement compared to the previous year is a performance test on gilts which was conducted at 27.747 gilts (23.208 in 2013). The increased number of tested gilts was recorded by Trivunovic et al. (2014), where they recorded a number of tested gilts in the last seven years, despite the fact that in 2011 the number of tested gilts fell by 3.3% in comparison to 2010. The positive growth trend continued in the following year by 6.6%.

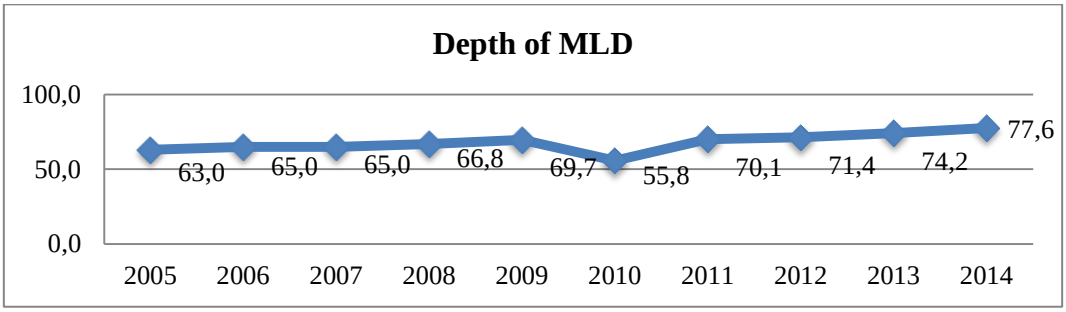

Chart 3. Depth of MLD in gilts in the test from 2005-2014.

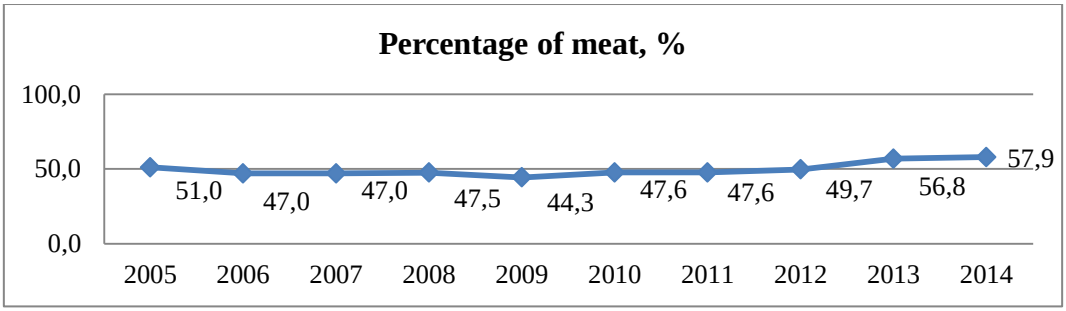

Chart 4. Percentage of meat in gilts in the test from 2005-2014.

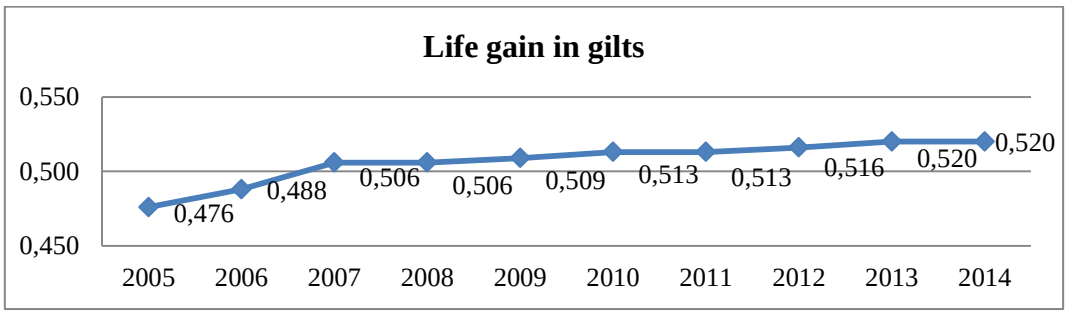

Chart 5. Life gain in tested gilts from 2005-2014.

By reviewing the results of the test it was determined an increase in the depth of MLD to 77.6 mm compared to the previous year (chart 3), which represented an increase of MLD compared to 2009, when the thickness of MLD was 69.7 mm (Radovic, 2010). The percentage of meat in 2014 was 57.9% (Chart 4), which represented an increase compared to

the percentage of meat in previous years. The reduction of the percentage of meat in the period from 2005-2009 was recorded by Radovic (2010) from 51% to 44.3%.
It was also an increase of live gain in tested gilts, which in 2005 amounted to 476 g and in 2008 506 g (Radovic, 2010). Increasing life gain in tested gilts was continued and in 2009 when the average life gain was 509 g, while in 2014 it amounted to 520g.

Conclusion

Swine production is an important part of agriculture in the overall economy of Vojvodina. On this basis, it is of great importance to increase the number of high-quality breeding animals and to improve the racial composition.
By specialized production on the national level, areas, with in a company (association) or large farms and by using modern methods of testing, selecting and crossing it is possible to improve the genetic basis of pigs in a relatively short period of time.
One example of the specialized production represents the organizational structure of breeding organizations in the Scandinavian countries, the hierarchical organization of the nucleus farm, production farms and commercial farms. Performance testing is done on gilts nucleus farms in Finland and production farms.
Reducing the number of tested boars is the result of strict selection, which is conducted in the choice of dam of boars, where strictly ensures that boars to be used for breeding improve the economic characteristics. Increasing the number of gilts at the end of the test is reflected in the possibility to perform ultrasonic measurement of the thickness of the back fat in a large number of gilts, with modern appliances by the Main breeding organizations. In order to done the appropriate selection of expectant mothers, and to increase the intensity of selection, it is included a larger number of gilts in the control.
Based on the results achieved in Vojvodina, leads to the conclusion that the implementation of Main breeding program by the Main breeding organizations produced a positive effect on the development of pig breeding in Vojvodina.
The objectives of the new breeding program has been expanded, so that they include new features (production, functionality, conformation), which are in accordance with professional standards, with optimal management (nutrition, medical care program and breeding animals), which will increase in the future and expand the positive effect implementation of breeding programs in pig production.

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CORRELATION BETWEEN BLOOD METABOLITES, MILK YIELD, DRY MATTER INTAKE AND ENERGY BALANCE IN DAIRY COWS DURING EARLY AND MID LACTATION

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Abstract

The objective of the present study was to investigate nutritional and metabolic status in Simmental cows during early and mid lactation. Fifteen early lactation cows and 15 mid lactation cows were chosen for the analysis. Blood samples were collected to measure beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), glucose and the activity of aspartate transaminase (AST). Blood metabolites, milk yield (MY), dry matter intake (DMI) and energy balance (EB) were recorded. Correlation analysis showed that DMI was significantly ($p<0.05$) negatively correlated with NEFA, BHB and AST, and positively ($p<0.05$) with glucose and TG. EB was significantly ($p<0.05$) negatively correlated with NEFA, BHB and AST, and positively ($p<0.05$) with glucose. Early lactation as compared to mid lactation cows were found to have significantly ($p<0.05$) higher blood serum concentrations of NEFA, BHB and AST activities and lower blood serum concentrations of glucose and TG, but not significant ($p>0.05$). These metabolic changes were in correlation ($p<0.05$) with DMI and EB, but not with MY($p>0.05$). Suggest that they can serve as useful indicators of the nutritional and metabolic status of dairy cows during lactation.

Keywords: *blood metabolites, dry matter intake, energy balance, dairy cows*

Introduction

Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows. Dairy cows suffer from negative energy balance (NEB) during the first weeks of lactation due to energy expenditure associated with milk production and limited feed intake, resulting high mobilization of lipids from body fat reserves, and hypoglycaemia (Veenhuizen et al., 1991; Drackley 1999, Oetzel, 2004; Djokovic et al., 2007,2011). Nutrition, age, heredity, body condition score (BCS), management and energy imbalance as various risk factors are possible causes of NEB, periparturient fatty liver and ketosis (Pechova et al., 1990; Drackley 1999). Clinical ketosis in dairy cows usually occurs between the second and seventh week of lactation. Nevertheless, most of cows in this stage of lactation may suffer a subclinical form of ketosis defined as increased blood ketone bodies without any other symptoms but accompanied by considerable decrease in milk yield and susceptibility other diseases (Duffield et al. 2000, Oetzel, 2004). The degree of NEB in early lactation and the recovery rate from are critical for health status and productivity. Various metabolic and endocrine blood and milk traits have been shown to relate to EB (Reist et al., 2002). Consequently, stressors and poor nutritional management causing reduction in voluntary DMI will result in large increases in NEFA around calving (Drackley, 1999). The main blood indicators of lipomobilization in ruminants are BHB, the most important and abundant ketone

body, and NEFA (Duffield et al. 2000, Oetzel 2004, González et al. 2011). NEFA are preferentially and greatly accumulated as TG in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes (Sevinc et al. 2003). However, when steatosis occurs, endogenous liver synthesis decreases, leading to a reduction in blood glucose, total proteins, albumins and globulins, cholesterol, TG and urea. (Veenhuizen et al. 1991, Sevinc et al. 2003, Oetzel 2004, Djokovic et al. 2007, 2011). Fatty liver infiltration and hepatocyte degeneration involve cell membrane damage and hepatocyte destruction coupled with the release of cytoplasm enzymes (AST, GGT, LDH) and marked increases in the circulating activities (Pechova et al. 1990, Drackley, 1999). The objective of the present study was to investigate nutritional and metabolic status in Simmental cows during early and mid lactation on the basis the correlations between blood biochemical metabolites, MY, DMI and EB**.**

Material and methods

Animals, diets and protocol design

This experiment was carried out in a dairy herd (166 Simmental cows) suffering from several metabolic and reproductive disorders. Two groups (n=15 cows) of clinically healthy cows were chosen from the herd. The cows were mid-yielding with a preceding lactation of about 6500 l and MY=25±5 l/day (early lactation cows: 6488±980, and MY=23±4 l/day); mid lactation cows cows: 6677±1088 and MY=28±6 l/day). Group 1 consisted of early lactation cows, in the first month of lactation (16 ± 9 days), and Group 2 included mid lactation cows between 3 to 5 months of lactation (114 ± 28 days). The experimental cows were kept in tie-stall barns. Diet and the housing facilities were adapted to research purposes, with diet suited to the energy requirement of early and mid lactation cows. Early lactation cows were fed a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% Dry Matter, DM), 5 kg concentrate (18% crude proteins, CP). Mid lactation cows received a diet consisting of 5 kg hay, 7 kg lucerne hay, 30 kg maize silage (30% DM), 8 kg concentrate (18% CP) Dietary nutrient contents for dairy cows in early and mid lactation are given in Table 1. The chemical analysis of the feed was performed by Weende methodology. EB was calculated by NRC recommendation (17).

Table 1: Nutrient contents in daily ration for early lactation and mid lactation dairy cows.

	Early lactation cows	Mid lactation cows
Dry matter intake (DMI) (kg)	16.05	24.82
Net Eanergy of Lactation (NEL) (MJ)	87.15	130.23
Crude Protein (CP) (% of DM)	13.58	13.38
Rumen undergradable protein (RUP) (% of CP)	35.91	28.33
Fat (% of DM)	3.09	3.14
Fiber (% of DM)	23.26	24.33
EB (MJ/day/cow) mean ± SD	-15,21 ±20.37	3.49 ±8.16

Biochemical analysis

Blood samples were collected at 10:00 h or 4 to 6 hours after milking and feeding, by puncture of the jugular vein into sterile disposable test tubes, without anticoagulant. After clotting for 3 hours at 4°C and centrifugation (1500g, 10 minutes, 4°C), sera were carefully harvested and stored at -20°C until analysis. Blood samples collected on fluoride were immediately centrifuged in the same manner and plasmas were assessed for glucose concentrations. The following biochemical blood components were measured at Biochemical Laboratory by different colorimetric techniques using spectrophotometers (Cobas Mira and

Gilford Stasar): BHB and NEFA levels were measured by Randox (United Kingdom) kit, AST and glucose by Human (Germany) kit, and TG by Elitech (France) kit.

Statistical analysis

Data were subjected to statistical analysis using the GLM model and t-test (Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, Va, Virginia, USA). The model included lactation period and metabolite values. Pearson's test was performed to evaluate significant correlations between MY, DMI, EB and biochemical metabolites separately. The intensity of change in metabolic profile during negative energy balance in early and mid lactation stages was analyzed by comparison of b parameters in linear equations (t-test). Finally, correlation between metabolic parameters was evaluated. Differences were considered significant at p values below 0.05 or 0.01.

Results and Disscusion

Blood biochemical metabolites MY, DMI and EB in early lactation and mid lactation cows were compared in this study. Homeorhesis induces intense lipid mobilization and ketogenesis, and the liver is adapted to metabolic changes in dairy cows. Intensive postpartal lipid mobilization and ketogenesis are sufficient for a series of compensatory metabolic processes with changes in blood metabolic profile during early lactation in healthy cows (Drackley 1999). Results on blood biochemical metabolites for both groups of cows are shown in Table 2.

Table 2.Blood metabolites in early and mid lactation dairy cows (n=15 in each group). Results are expressed as mean ± standard deviation (SD).NS: non-significant

	Early lactation cows	Mid lactation cows	p
Glucose (mmol/l)	2.29±0.48	2.76±0.43	< 0.05
BHB (mmol/l)	1.59±0.25	0.91±0.16	< 0.05
NEFA (mmol/l)	0.38±0.29	0.13±0.04	< 0.05
TG (mmol/l)	0.12±0.02	0.15±0.04	NS
AST (U/l)	69.46±27.54	39.31±18.90	< 0.05

The metabolic levels significant correlated with DMI and EB and did not significant correlated with MY (Table 3).

Table 3. Correlation coefficients between metabolites and milk production, DMI and EB. Significant correlations ($p<0.05$) are marked with asterix (*).

Metabolites	MY(l/day)	DMI (kg/day)	EB (MJ/day)
Glucose	r= 0.18	r= 0.43*	r= 0.45*
NEFA	r=-0.21	r=-0.50*	r=-0.49*
BHB	r=-0.25	r=-0.38*	r=-0.53*
TG	r=-0.11	r= 0.42*	r= 0.18
AST	r= 0.07	r=-0.49*	r=-0.41*

Change of metabolites as a function of EB in early and mid lactation is shown in Table 3. The values changed much more intensely in early lactation than in mid lactation. The decrease in energy balance led to increased NEFA, BHB and AST and decreased glucose levels for every unit change much more intensely in early lactation than in mid lactation.

Table 4. Change of metabolites as a function of EB in early and mid lactation (comparison of b parameters from linear equation). NS: non-significant

	b parameters	(relation EB to metabolite)	
Metabolites	Early lactation	Mid lactation	p
Glucose	0.08	0.05	<0.05
NEFA	-0.07	-0.03	<0.01
BHB	-0.09	-0.05	<0.01
TG	0.002	0.003	NS
AST	-4.35	-2.28	<0.01

The correlation coefficients among the biochemical parameters calculated for all cows in this experiment are summarized in Table 5.

Table 5**.** Correlation coefficients for the biochemical metabolites calculated for all cows in the present study. Significant correlations ($p<0.05$) are marked with asterix (*).

	NEFA	BHB	TG	AST
Glucose	r= -0.35*	r=-0.47*	r=0.65*	r=-0.23
NEFA		r=0.39*	r=-0.21	r= 0.34*
BHB			r=-0.36*	r=0.15
TG				r=-0.04

In early lactation cows, NEFA and BHB values were significantly higher than in mid lactation cows. NEFA concentrations > 0.40 mmol/l indicate problems with energy balance and subsequent intensive lipomobilization (Oetzel, 2004). According to this report, in early lactating cows, NEFA values in blood were 0.38 ± 0.29 mmol/l, showing evidence of high lipomobilization in the present study. These are result the some early lactating cows in the present study had NEFA concentrations above the value indicative of subclinical ketosis. Subclinical ketosis also may be diagnosed when serum BHB concentrations are above 1.2 mmol/l, while clinical ketosis is associated with BHB concentrations above 2.6 mmol/l (Duffield 2000, Oetzel, 2004). These are result early lactating cows in the present study had BHB concentrations above the value indicative of subclinical ketosis (1.59±0.25 mmol/l). The data presented show that serum NEFA may be used for detecting high lipomobilization, but not subclinical ketosis. This is in agreement with Duffield (2000), who stated that the use of NEFA is a better indicator of energy imbalance in prepartum animals than BHB, but BHB is more useful postpartum. In the present study, a significant positive correlation was established between NEFA and BHB in the sera, suggesting that both parameters are helpful indicators of energy balance during lactation. Blood NEFA and BHB concentrations were not in correlation with the intensity of milk production during lactation, but a significant correlation was observed with DMI and EB. Reist et al. (2001) reported a strong correlation among blood NEFA and BHB concentrations and EB in early lactation dairy cows. BHB is negatively correlated with food level and it therefore increases in cows fed a good diet in dry and lower quality in post partum (Stockdale, 2008). Blood glucose values in mid lactation cows were within the physiological range 2.5 - 4.2 mmol/l (Oetzel 2004), whereas hypoglycemia (2.29 ± 0.48 mmol/l) was detected in early lactation cows. Taking this criterion into account, early lactation cows had indicative values, but did not display any clinical signs, suggesting that they had a typical subclinical condition. Blood glucose concentrations in early lactation cows were significantly correlated with DMI and EB. In fact, a significant correlation was observed between NEFA values and glucose and BHB and glucose. Similar correlations were observed by other authors (Reist et al. 2002, Laszlo et al. 2009). Cows in NEB have low rates of glucose and high levels of BHB (González et al. 2011). Fat infiltration into the liver may also affect the concentration of some blood components. Serum level of TG, is indicator of hepatic

functionality, and decreases in their concentration may suggest fat infiltration in the liver (Sevinc et al. 2003, Djokovic et al. 2007, 2011). The concentration of serum TG was significantly lower in ketotic cows compared to healthy cows (Djokovic et al. 2007). These results are showed that TG accumulate in the liver cells of ketotic cows and cause their blood values to decrease. In the present study, TG in the blood was low (0.12 ± 0.02 mmol/l vs 0.15 ± 0.04 mmol/l) in both groups of cows, but without significant difference. This study was showed a posibility of the development a fat infiltration of the liver in early lactation cows and was confirmed a significant correlation between TG and glucose, TG and DMI and TG and BHB. When fat infiltrates the liver, a lesion appears in hepatic tissues, and the levels of enzymes that indicate liver injury (AST, GGT, and LDH) are generally augmented (Pechova et al. 1990). AST values in the present study were statistically higher ($P<0.05$) in early lactation cows than in mid lactation cows. If AST activity higher than 100 U/l is indicative of hepatic lesions (González et al.2011). These are result early lactation cows in our study suffered from some degree of hepatic lesions, probably due to fat infiltration. Also, a positive correlation was observed between AST activity and NEFA values. In the present study, all data concerning serum AST activities suggested that the process of lipomobilization was sufficient to cause liver lesions in of the early lactating cows. These metabolic changes were not in correlation with the MY during lactation, but a significant correlation was observed between metabolite values and DMI and EB. According to Wathes et al. (2007), milk yield was independent to nutritional and biochemical parameters during only the first two weeks post-calving but it was significantly higher in cows expressing high levels of BHB and urea at the 4th week. Lower DMI could explain lower concentrations of glucose and TG, and higher concentrations of NEFA, BHB and AST. Negative energy balance could explain lower glucose concentrations and higher NEFA, BHB and AST levels. Insufficient DMI in early lactation is known to lead to NEB with the above mentioned changes occurring in the metabolic profile. Metabolic changes during early lactation were more intensive as a function of EB, compared to mid lactation. These changes are due to both lipolysis and ketogenesis occurring during homeorhetic processes and metabolic adaptation in the liver during early lactation. Several authors reported these changes but with different intensities (Reist et al. 2002, Laszlo et al 2009).

Conclusion

On the basis of changes of blood biochemical metabolites, this study suggests that early lactation cows had metabolic disturbances, which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. These metabolic changes were correlated with DMI and EB, but not with MY, and they can serve as useful indicators of the nutritional and metabolic status of dairy cows during lactation.

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EGG WEIGHT AND SHELL QUALITY CHARACTERISTICS OF LAYING HENS FED WITH GRADED LEVELS OF COCOA BEAN SHELL

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Abstract

Thirty (30), twenty – three weeks old Isa brown pullets were used to investigate the egg weight and shell quality characteristics of laying hens fed with different levels of cocoa bean shell. The cocoa bean shell was used to replace maize at 0%, 25%, 50%, 75%, and 100% levels of inclusion. The replacement levels of 0% represent the control treatment (treatment 1). Each diet was fed to the birds for 56 days (8 weeks) and the investigation was on fed intake and egg components which are shell weight, egg weight and shell thickness.

The increase in the inclusion of cocoa bean shell in the diet has no significant effect ($P>0.05$) on the shell weight and shell thickness. However, feed intake decreased with increase in the inclusion level of CBS, and this contributes to the lower body weight of the birds with dietary treatment above 25% CBS meal. At 25% CBS inclusion, the egg weight and the feed intake of the birds are still the same or close to the control treatment. The egg weight of the birds fed with dietary treatment above 25% CBS meal decreases which shows that bird fed with CBS at 25% inclusion level can still have a desirable egg weight.

This investigation shows that CBS meal could partially replace maize up to 25% in layers' diet without negatively affecting the egg weight and shell quality characteristics of the eggs.

Keywords*:* *Cocoa Bean Shell, Isa Brown Pullets, Egg Quality Characteristics.*

Introduction

Monogastrics, particularly poultry compete well with man for conventional feed stuff such as cereal, grains and legumes. This competition between man and livestock has led to scarcity and high cost of feed ingredients and this has resulted in high cost finished foods of animals products which are beyond the reach of average consumers. In Nigeria in recent years, the once prosperous poultry industry has virtually collapsed due to shortage and high cost of feed ingredients particularly maize. Since commercial feed in the country contain an average of 50% maize and 90% of the feeds are consumed by poultry (Olayemi, 1989). However this problem is being aggravated by the ever-increasing demand for maize as both staple food for man and an industrial raw materials, as the country has no recourse to cheap imports or increased supply of maize, the option has been to source locally for available alternative plant materials which are not components of man's food. The high cost of maize grains necessitates the search or locally available materials like agro-industrial by products and wastes, with a view of finding those that could supplement the ration at different levels which would lower feed cost without sacrificing efficiency of livestock production.

However, Egbunike and Ikpi, (1988) highlighted the tremendous potential of agro-industrial by-products which were at least 738,271.6 tonnes and crop residues of at least 52 million tones in Nigeria. Ogbonna (1991) confirmed that production of agro-industrial waste is at least one million tonnes per annum in the same country. The potential valve of agro-industrial by-products and the maximum inclusion rates in livestock diets depend on their nutritional characteristics, their safety for animal health (Boucque and fiems, 1988). Some examples of these agro-industrial by-products are palm kernel, cocoa pod husk, cocoa bean shell, cocoa dust, cocoa bean cake, brewery dry grains (BDG), Wheat bran, e.tc. and these have been

proven to be useful in alleviating the high cost of conventional feed ingredients especially maize. On the other hand, cocoa by-products such as cocoa bean cake, cocoa pod-dusk, cocoa bean shell cocoa butter, discarded cocoa beans etc. all have a theobromine content which has contributed to their limited use in farm animal feed. Theobromine, when taken in moderate quantities acts as a stimulant like caffeine but intake of more the 0.0279kg-1 body weight is injurious to animals. (M.A Menon 1982; Gohl 1981). Cocoa by-products are also high in nutritive value for livestock feed. (M.A. Menon 1982; Gohl 1981).

Cocoa bean shell, (CBS) constitutes nearly 10% of cocoa bean (Menon, 1982). It contains 17.5% crude protein, 13%fibre, 8.1% ash and an anti-nutritional component, theobromine, which is of the proportion 1.3 – 2.0% (Menon, 1982. Yeong*et al.*, 1989; Abiola and Tewe, 1991). It is a by-product of industries processing low grade cocoa beans. It is an unconventional feed ingredients that could be utilized by poultry and is not consumable by man. Gohl (1981) reported that CBS is high in nutritive value but it is of limited use in animal feeds (livestock) because of its theobromine content (anti-nutrient). However there are simple, cheap and effective means of reducing and removing the theobromine content in CBC. Theobromine, when taken in moderate qualities acts as a stimulant like caffeine, but intake of more than $0.0279kg^{-1}$ body weight is injurious to animals (Menon, 1982).

The aim of this paper is to determine the egg weight and shell quality characteristics of layer birds fed with different inclusion level of cocoa bean shell, substitute with maize in the layers mash.

Material and methods

Experimental site

The experiment was carried out at kings farm at Apata, Ibadan, Oyo State Nigeria during 2002. The cages were properly cleaned, Water troughs and the feeding troughs were washed and disinfected a week before the arrival of the birds.

Experimental diet

The cocoa bean shell used for this research was collected from Stanmark Cocoa Processing Company Limited km5 Ondo-Akure roads, Ondo State, Nigeria.

The cocoa bean shell was used to replace maize at 0%, 25%, 50%, 75%, 100% levels of inclusion. The percentage composition of the experimental diets are presented in a table 2.1

Table1. Gross composition of experimental diets

Ingredients (%)	**T1**	**T2**	**T3**	**T4**	**T5**
Maize	40.00	30.00	20.00	10.00	-
Cocoa bean shell	-	10.00	20.00	30.00	40.00
Brewery dried	12.00	12.00	12.00	12.00	12.00
Grain					
Wheat bran	4.00	4.00	4.00	4.00	4.00
Palm Kernel cake	7.00	7.00	7.00	7.00	7.00
Soya meal	21.00	21.00	21.00	21.00	21.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Bone meal	5.00	5.00	5.00	5.00	5.00
Oyster shell	5.00	5.00	5.00	5.00	5.00
Layer premix	0.25	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100	100

Calculated values

Crude protein	16.75	17.19	17.64	18.29	18.51
ME (Ka1/kg)	2558	2456	2354	2252	2150
Crude fibre	6.3	6.35	6.41	6.70	7.01
Ether Extract	3.93	4.22	4.30	4.33	4.39
Dry matter	90.00	90.29	91.63	91.63	91.82

Analyzed values

Crude protein	16.75	16.85	17.04	17.04	17.40
Crude fibre	6.30	6.35	6.41	6.41	6.50
Fat and Oil	3.93	4.22	4.30	4.30	4.39
Ash	8.86	9.91	10.16	10.00	10.03
Nitrogen free extract	64.16	62.67	62.09	62.25	61.68
Dry matter	90.00	90.29	91.06	91.03	90.42
GE (Kcal/kg)	3452.80	3428.00	3403.70	3400.40	3397.40

Layer premix composition (content per 2.5kg)

Vitamin A	10,000iu
Vitamin D_3	20,000,00iu
Vitamin E	10,000iu
Vitamin K	1.20g
Thiamine B_1	1.30g
Riboflavin B_2	4.00g
Niacin B_3	13.00g
Pyridoxine B_6	1.25g
D-Calpan B_5	5.00g
Biotin	0.02g
Folic acid	0.40g
Vitamin B_{12}	0.10g
Manganese	50.00g
Zinc	45.00g
Copper	7.00g
Iron	50.00g
Iodine	1.00g
Cobalt	0.30g
Selenium	0.10g
Choline Chloride	250.00g
BHT	125.00g

Table 2. proximate composition of cocoa bean shell (%DM)

Constituents (%)	**CBS**
Dry matter	98.07
Crude protein	13.12
Crude fibre	13.00
Ether extract	8.71
Ash	9.15
NFE	56.0
ME (kcal1kg)	24.00

Experimental bird and management

Thirty (30), Twenty-three weeks old Isa brown pullets were randomly allotted to 5 dietary treatment with two replicated per treatment. Six birds were allocated to each treatment with three (3) birds per replicate. Eight (8) weeks feeding trial was conducted to determine the optimal substitution level of cocoa bean shell for maize in laying hens.The cages we properly cleaned, water troughs and the feeding troughs were washed and disinfected a week before the arrival of the birds. Fresh feed and water were provided ad-libitum. The feed remnants (left over) were removed and the troughs cleaned before serving another feed.

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) Procedure (steel & torrie, 1980) while treatment means were separated by Duncan Multiple Range Test (Gomes and Gomes, 1985).

Results and discussion

Table 3.egg weight and shell quality characteristic of laying hens fed with cocoa bean shell.

Parameter	T1 0% CBD	T2 25% CBS	T3 50% CBS	T4 75% CBS	T5 100% CBS	SEM
Ave. daily feed intake (g/bird/day)	99.74[a]	95.52[b]	82.71	80.28	70.95[d]	1.07
Egg weight (g)	59.56[a]	59.37[a]	59.60[b]	52.44	50.22[d]	0.66
Shell weight (g)	6.18	6.17	6.11	6.08	6.07	0.11
Shell thickness (mm)	0.36	0.36	0.35	0.34	0.37	0.10

SEM – Standard Error of Means

Row means with different superscript are significantly different [P<0.05]

Feed intake: The feed intake result presented in table 3.1 shows that’s there is significant difference in feed consumption of hens fed different levels of CBS meals (P<0.05). Feed intake of the birds reduced as the level of CBS increases. This decrease in feed intake is as a result of theobromine content (1.3%) in the CBS, which is toxic to the animal. And this is in agreement with Menon (1982) that intake of more than 0.027g/kg is injurious to animal. This can lead to depressed feed intake and consequently reduce performance (Olubamiwa, *et al.,* 2000; Odunsi, *et al.,*1999 and Yeonge*t al.,* 1989). The reduction in the feed intakeleads to lower body weight.

Feed Intake (g/bird/day)

Week	T1	T2	T3	T4	T5
1	98.50	96.25	84.60	79.26	68.44
2	100.25	94.25	82.70	79.95	69.99
3	96.50	93.10	80.56	80.28	70.85
4	99.50	98.60	80.64	90.56	69.96
5	102.50	91.50	82.90	70.14	69.96
6	99.70	99.60	82.49	80.98	70.85
7	100.30	95.22	83.90	79.79	74.24
8	100.20	98.14	83.89	81.29	71.64
Total	797.90	767.36	661.68	642.24	567.60
Mean	99.74	95.92	82.71	80.28	70.95

Analysis of variance table

	Degree of freedom	Sum of Squares	Mean Square	F-value	Probability
Between 0.0000	4	4456.991	1114.248	121.046	
Within	35	322.180	9.205		
Total	39	4779.171			

Coefficient of variation = 3.53%

Var

1	Number	Sum	Average	SD	SE
1	8.00	797.900	99.738	1.72	1.07
2	8.00	767.360	95.920	2.79	1.07
3	8.00	661.680	82.710	1.48	1.07
4	8.00	642.250	80.281	5.50	1.07
5	8.00	567.600	70.950	1.69	1.07
Total	40.00	3436.790	85.920	11.07	1.75
Within				3.03	

Duncan’s multiple range tests

Original Order	Ranker Order
Mean 1 = 99.74 A	Mean 1 = 99.74 A
Mean 2 = 95.92 B	Mean 2 = 95.92 B
Mean 3 = 82.71 C	Mean 3 = 82.71 C
Mean 4 = 80.28 C	Mean 4 = 80.28 C
Mean 5 = 70.95 D	Mean 5 = 70.95 D

Egg weight: table 3.1 shows that there significant difference in egg weight of laying hens fed different levels of CBS meal ($P<0.05$). It can also be deduced that there is no significant difference between the control treatment (0%CBS inclusion) and treatment 2 (25%CBS inclusion). The result shows that there is a significant difference between treatment 2,3,4 and 5. At 50% inclusion of CBS the egg weight decreased significantly ($P<0.05$) which shows that bird fed with CBS at 25% has a desirable egg weight that those fed with dietary treatment.The decrease in egg weight of birds fee CBS correlate with the report of Gohl (1981) and Hutagalung and Chang (1978) who reported that low level of dietary CBS enhance egg quality but high level limits egg quality due to its theobromine content (anti-nutrient factor)

Egg weight (g)

Week	T1	T2	T3	T4	T5
1	54.96	57.66	59.60	50.20	48.75
2	58.63	59.10	54.98	49.84	50.12
3	60.28	61.59	58.20	52.44	50.12
4	60.58	62.10	56.78	53.63	52.68
5	60.69	61.29	56.14	50.12	49.59
6	60.25	58.74	56.37	54.43	50.16
7	60.28	58.76	57.35	54.43	49.38
8	60.54	55.722	55.38	54.43	50.96
Total	476.48	474.96	452.80	419.52	401.76
Mean	59.56	59.37	56.60	52.44	50.22

Analysis of variance table

	Degree of Freedom	Sum of Squares	Mean Square	F- value	Probability
Between 0.0000	4	558.538	139.635	40.454	
Within	35	120.810	3.452		
Total	39	679.348			

Coefficient of Variation = 3.34%

Var

1	Number	Sum	Average	SD	SE
1	8.00	476.480	59.560	1.98	0.66
2	8.00	474.960	59.370	2.18	0.66
3	8.00	452.800	56.600	1.68	0.66
4	8.00	419.520	52.440	2.09	0.66
5	8.00	401.760	50.220	1.19	0.66
Total	40.00	2225.520	55.638	4.17	0.66
Within				1.86	

Duncan’s multiple range tests

Original Order	Ranked Order
Mean 1= 59.56 A	Mean 1 = 59.56 A
Mean 2 = 59.37 A	Mean 2 = 59.37 A
Mean 3 = 56.60 B	Mean 3 = 56.60 B
Mean 4 = 52.44 C	Mean 4 = 52.44 C
Mean 5 = 50.22	Mean 5 = 50.22 D

Shell weight: The shell weight results presented in table 3.1 shows that there is no significant difference between the shell weight of eggs layers fed different levels of CBS meals ($P<0.05$). This indicates that increase in the inclusion of CBS meal in diet of layers has no effects on the shell weight.

Shell weight (g)

Week	T1	T2	T3	T4	T5
1	5.70	5.99	6.43	5.82	5.89
2	5.86	6.14	5.93	5.78	6.06
3	5.67	6.40	6.28	6.08	6.06
4	6.10	6.45	5.91	6.22	6.37
5	5.80	6.37	6.06	5.81	6.00
6	6.45	6.10	6.08	6.31	5.97
7	6.75	6.11	6.19	6.31	5.97
8	7.10	5.29	5.98	6.31	6.16
Total	49.43	49.35	48.86	48.64	48.57
Mean	6.18	6.17	6.11	6.08	6.07

Analysis of variance table

	Degree of Freedom	Sum of Square	Mean Square	F- value	Probability
Between 0.0000	4	0.080	0.020	0.223	
Within	35	3.130	0.089		
Total	39	3.210			

Coefficient of variation = 4.89%

Var

1	Number	Sum	Average	SD	SE
1	8.00	49.430	6.179	0.53	0.11
2	8.00	49.350	6.169	0.23	0.11
3	8.00	48.860	6.107	0.18	0.11
4	8.00	48.640	6.080	0.24	0.11
5	8.00	48.570	6.071	0.14	0.11
Total	40.00	244.850	6.121	0.29	0.05
Within				0.30	

Duncan’s multiple range tests

Original Order	Ranked Order
Mean 1 = 6.180 A	Mean 1 = 6.180 A
Mean 2 = 6.170 A	Mean 2 = 6.170 A
Mean 3 = 6.110 A	Mean 3 = 6.110 A
Mean 4 = 6.080 A	Mean 4 = 6.080 A
Mean 5 = 6.070 A	Mean 5 = 6.070 A

Shell thickness: The result also shows that different dietary levels of CBS meals does not have significant effects on shell thickness of eggs ($P<0.05$). This means that increase in the inclusion of CBS meal has no effect on the thickness of egg shell, although shell thickness tend to decrease slightly with increasing level of CBS meal.

Shell thickness

Week	T1	T2	T3	T4	T5
1	0.34	0.31	0.36	0.37	0.35
2	0.36	0.43	0.36	0.35	0.33
3	0.37	0.32	0.34	0.33	0.32
4	0.37	0.35	0.33	0.31	0.33
5	0.36	0.36	0.37	0.35	0.32
6	0.36	0.37	0.34	0.33	0.30
7	0.33	0.34	0.36	0.34	0.34
8	0.39	0.41	0.34	0.34	0.35
Total	2.88	2.89	2.80	2.72	2.64
Mean	0.36	0.36	0.35	0.34	0.33

Analysis of variance table

	Degree of Freedom	Sum of Square	Mean Square	F-value	Probability
Between 0.0619	4	0.006	0.001	2.479	
Within	35	0.020	0.001		
Total	39	0.026			

Coefficient of variation = 6.88%

Var

1	Number	Sum	Average	SD	SE
1	8.00	2.880	0.360	0.02	0.01
2	8.00	2.890	0.361	0.04	0.01
3	8.00	2.800	0.350	0.01	0.01
4	8.00	2.720	0.340	0.02	0.01
5	8.00	2.640	0.330	0.02	0.01
Total	40.00	13.930	0.348	0.03	0.00
Within				0.02	

Duncan’s multiple range tests

Original Order	Ranked Order
Mean 1 = 0.3600 A	Mean 1 = 0.3600 A
Mean 2 = 0.3600 A	Mean 2 = 0.3600 A
Mean 3 = 0.3500 A	Mean 3 = 0.3500 A
Mean 4 = 0.3400 A	Mean 4 = 0.3400 A
Mean 5 = 0.3300 A	Mean 5 = 0.3300 A

Conclusion

The result obtained from the experiment indicate that birds fed with dietary treatment 2 (25% CBS inclusion) showed no significant difference ($P<0.05$) with the control (0% CBS inclusion) with respect to feed intake, egg weight, hell weight and shell thickness.

The increase in the inclusion level of CBS meal also has no significant effect on the shell weight and shell thickness across the treatment.

However treatment 1 and treatment 2 results are significantly higher than other treatments with respect to feed intake, egg weight, shell thickness and shell weigh. It is therefore concluded that laying hens can tolerate 25% replacement of maize with CBS meal and

produce eggs with desirable weight and shell qualities economically, so also with a good body weight.

Based on the results of this experiment, 25% cocoa bean shell meal is recommended for replacement of maize in the feed of laying birds.

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EFFECTS OF SUBSTITUTION OF SOYBEAN MEAL BY FABA BEAN ON FATTENED HOLSTEIN BULLS PERFORMANCES IN FINISHING PHASE

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Abstract

The current research aimed to study the effect of substituting soybean meal by faba bean on performances of fattened Holstein bulls in finishing phase. This substitution concerns 50% and 100% of the nitrogen (N) content in a conventional concentrate provided by soybean meal, with another source of N as faba bean. Authors used 33 Holstein bulls divided into 3 homogenous groups (live-weight and age averaged 346.5 kg and 13.5 months, respectively). The basal diet was composed of oat hay and oat silage, supplemented with 3 types of isoenergetic and isonitrogenous concentrates containing 0% $N_{faba\ bean}$ (D1), 50% $N_{faba\ bean}$ (D2); 100% $N_{faba\ bean}$ (D3) as substitute of N provided by soybean meal. The trial lasted 3 months, and animals were weighted every 2 weeks, and intake measurements were carried out during 3 successive days, in the middle of the period between each 2 weighing times. The results showed that total conversion ratio was significantly ($P< 0.001$) higher for animals receiving concentrates with partially or totally (D2 and D3: averaged 10.27) substituted N than the conventional one (D1: 10.92). No differences were noted in total dry matter intake between the 3 groups (averaged 12.26 kg DM/day). Furthermore, the average daily gain (ADG) resulting from the total substitution of N (D3), was significantly ($P< 0.05$) higher (1.24 g/day) comparatively to the other diets. The including of faba bean did not result in any changes in the final live-weight of animals. Also, the study showed that the substitution of soybean meal by faba bean, particularly once produced by farmers, leaded to a decrease in the feeding cost of the kg of gain (1.46 USD, 1.42 USD, 1.07 USD, respectively in D1, D2 and D3). It was concluded that in the current experiment conditions, substituting soybean meal with faba bean on the base of N supply resulted in higher performances and lower cost for meat production.

Keywords: *Faba bean minor, soybean, Bulls, Fattening*.

Introduction

Bovine meat production holds a great importance in Tunisian animal production. It represents 47% of total meat production (Mehiri, 2010). However, this activity is suffering from several technical and economical constraints resulting in low or moderate performances and high costs. In livestock production, feeding costs represent 60-80% of total charges (Rejeb Gharbi et al., 2007) ;moreover, unpublished investigations carried out in our laboratory showed that concentrate represents about 70% of feeding charges, corresponding to 60% of the total cost of meat. This situation is more and more accentuated by the growing of raw material international prices, mainly soybean and corn grain. Also, this is associated with the use of low quality basal diets overall made of straw and oat hay. Consequently, in order to limit this dependence on imported products, farmers have tested several alternatives to expensive proteic and energetic sources. So they substitute soybean meal (SBM) by some local proteaginous crops as faba bean (*Vicia faba*). In some Tunisian regions, Faba bean grains are traditionally used for bulls fattening especially during the finishing phase. The current work aimed to study the effect of partial or total substitution of soybean meal by faba bean on bulls' intake and performances.

Materials and methods

Animals

The trial was carried out from Mars to June 2009, in the farm of Loubira (North West of Tunisia, sub humid zone). We used 33 Holstein bulls in finishing stage of fattening (average age and initial live weight 13.5 months and 346.5 kg respectively). They were divided into 3 homogenous groups and housed in blocked stalling equipped with linear mangers and troughs. The nutritional requirements of animals were calculated to reach an average daily gain (ADG) equal to 1200 g/day (INRA, 1981).

Feed and diets

All animals received common basal diet (3 kg oat hay and oat silage *ad libitum*), and supplemented with 3 types of fattening concentrate iso-energetic and iso-nitrogen: C0: 0% faba bean (D1), and 2 others concentrate in which the substitution of soybean meal is partial C50 (50% of crude protein (CP) are brought by faba bean: D2) or total C100 (100% of CP are brought by faba bean: D3).

The basal diet was distributed twice /day and the concentrate is distributed 3 times/day at 8 a.m, 12 a.m and 4 p.m. The quantity of concentrate was about 2% of live weight, and is adjusted every 2 weeks after the weighing of bulls.

The raw materials used for the formulation of concentrated food were soybean meal, corn, barley, sound of wheat and the faba bean minor. The centesimal composition and the feeding value of three tested concentrated food are detailed in table 1.

Table 1. Centesimal Composition of the three experimental concentrate.

	faba Bean	soybean 46	corn	barley	Sound wheat	CaCo3	Ph bCa	salt	premix
C0	-	15	10	40	30	1.75	2	0.75	0.5
C50	14.5	7.5	10	43	20	1.75	2	0.75	0.5
C100	28	-	18	20	30	1.5	1.5	0.5	0.5

Measurements and calculation

This essay lasted 3 months. It starts with a period of adaptation of 10 days. Bulls are weighed every 15 days, to adjust the quantities of concentrate and began a period of measure of the ingestion for 3 successive days.

Along the study some samples of concentrate were collected and tow samples of roughage feed were collected for analysis of dry matter (DM), ash, CP, crude fiber (CF) according to AOAC (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed as described by Goering and van Soest (1970).

Dry matter intake of every feed, and of total dry matter intake, were calculated, as well as the ADG and the index of consumption, for various period of measure and global for all the phase of fattening.

Statistics

These measures were subjected to the statistical analysis by the procedure ANOVA of the system SAS (1988). The effect of the type of concentrate was studied through the effect of the experimental diet (D) on the various zootechnic performances measured, according to the following model: $Y = \mu + Di + Pj + D * P + Eij$

With, D: diet effect; P: period effect; D*P: interaction between effects of diet and period; E: error.

Results and discussions

Chemical composition of feeds

The real chemical composition of raw materials used in the manufacturing of the experimental concentrate food is given in table 2. And the real chemical composition of the roughage constituting the basic ration and experimental concentrate is given in the table 3.

The used oat hay is weak in CP and ash (respectively, 4.1 % DM and 6.8 % DM), corresponding to the hay used generally in Tunisia (Kraeim et al., 1997; Moujahed et al., 2011); but the content in CP of the oat silage is low (5.5 % DM) with regards to the bibliography (Mc Cartney et Vaage, 1993; Moujahed et al., 2009). For the NDF and the ADF content are similar to those found by (Mc Cartney et Vaage, 1993). For the concentrate, particularly the content in CP is relatively similar between the various types of concentrate.

Table 2. Chemical composition of raw materials (%DM).

	%DM	ash	CP	CF	NDF	ADF	ADL
Corn	86.5	1.3	7.8	1.54	82.6	2.8	0.5
Faba bean	89.5	3.6	26.1	10.44	37.4	12.3	0.2
Soybean meal 46	87.8	6.8	37.35	6.39	8.7	5.6	0.8
barley	89.8	2.9	9.8	6.0	43.4	9.0	1.0
Sound of wheat	86.2	5.2	14.37	7.67	39.1	12.4	3.0

Table 3. Chemical composition of roughage feeds and experimental concentrate (% DM).

	%DM	ash	CP	CF	NDF	ADF	ADL
Oat silage	22.5	14.6	5.5	-	58.5	36.5	4.1
Oat hay	85.7	6.8	4.1	-	70.7	43.6	5.4
Conc. C0		8.4	17.7	5.4	34.4	7.2	1.1
sd C0		1.6	0.4	1.1	4.7	0.8	0.1
Conc. C50		7.6	16.9	5.7	31.7	8.0	0.8
sd C50		0.9	0.7	1.1	4.0	1.3	0.2
Conc. C100		7.6	16.2	7.0	33.9	8.3	1.0
sd C100		0.7	1.5	1.0	2.4	0.6	0.2

Intake Parameters

Table 4. Intake parameters for different experimental regimes.

	D1	D2	D3
DMI o. Silage *	4.11 a	4.18 b	4.34 b
DMI o. Hay *	1.81 a	1.69 b	1.67 b
DMI conc	6.34	6.30	6.35
Tot DMI	12.26	12.17	12.36
DMI/kg $P^{0,75}$	0.134	0.135	0.135
IC	10.73	10.49	9.8
Global-IC ***	10.92 A	10.42 B	10.13 B

*: (P<0.05); ***: P<0.001

Intake parameters presented in table 4, show that the quantity of DMI of oat silage is relatively higher (P < 0.05) for D2 group and D3 group (average 4.26 kg DM) than the D1 one (4.11 kg DM). On the other hand, the DMI of oat hay is a little bit lower (P< 0.05) for the groups D2 and D3 than the group D1. It seems that the incorporation of faba bean contributes to a light decrease of the most cumbersome feed, of made of that the faba bean is richer in

walls than the barley and that his proteic /bulk report is more raised than the meals (RAD, 2006).
Besides, there is no significant difference between groups for the intake of the total dry matter, and that of the intake of the dry matter of concentrate. Moreover, the global ratio of consumption is relatively higher (P< 0.001) for D1 group (10.92) with regards to the D2 and D3 groups (respectively 10.42 and 10.13). Our results are in accordance with several works, such as of Haurez (2002) by substituting totally the soybean meal by the faba bean for the fattening of the Frisian bull-calfs with the corn silage. Also, for a regime with oat hay in the works of Ben Salem and al. (2006) by total substitution of soybean meal by the faba bean, and Moujahed and al. (2008) by substituting partially the soybean meal (16 %, 23 % of faba bean). These authors did not find significant differences for dry matter intake and the ratio of consumption between the soybean meal and the faba bean.
So authors conclude that the partial or total substitution of soybean meal by the faba bean in the fattening of bull-calfs in finishing phase, reduce relatively the consumption of hay of whose the UF is more expensive and increases the intake of silage which is more economic. While considering that the intake of concentrate is the same. On the other hand, the concentrate with faba bean diminish relatively the index of consumption (IC) showing that the introduction of faba bean enable to enhance the value of the daily feed ration by animals. Furthermore, economically the substitution of soybean meal by faba bean, particularly once produced by farmers, leads to a decrease in the feeding cost of the kg of gain (1.46 USD, 1.42 USD, 1.07 USD, respectively in D1, D2 and D3.

Growth’s performances of animals

The effect of feeding regime on performances of bulls as change of live weight LW (kg) and ADG are presented in table 5.

Table 5. Growth’s parameters of fattened bulls depending on different regimes.

	D1	D2	D3
initial LW (Kg)	370.9	365.4	368.4
Final LW (Kg)	482.45	477.3	488.6
LW gain	111.54	111.94	120.28
P-ADG	1.150 b	1.170 b	1.240 a

*: P< 0.05; P-ADG: pondered ADG.

In the term of this essay, we found that the total substitution the soybean meal by the faba bean in the D3, relatively engendered a better performance of growth to the bull-calves of the D3 group (table 5, figure 1). Indeed, their P-ADG = 1.24 kg, is slightly more raised (P < 0.05) than the P-AGD engendered by the other regimes D1 and D2, respectively (1.15 kg and 1.17 kg) and which are not significantly different from each others.

But, for other growth parameters (initial weight (IW), final weight (FW), LW gain there is no significant difference between the various diets. The improvement of weight gain is about 90 g, in favour of diet with Faba bean. While, the partial substitution (Moujahed et al., 2008) or the total substitution (Ben Salem and Fraj, 2006) of the soybean meal by the faba bean did not engender a significant variation of the AGD, it is probably due to their diet based on oat hay. Indeed, the energetic density of the silage is better allowing an improvement of the gain of the animals’ weight (Sansoucy et al., 1984; Micol et al., 2003), relatively rich in proteins especially after a phase of feed restriction (Micol et al., 2003). Moreover, the nitrogenous retention is better for the silage than the hay (Smeti et al., 2012), even if, the silage used in this trial is low in nitrogen. Other part, the faba bean is especially rich in non-protein nitrogen

fraction which is very soluble and easily degradable and represents 12 % of CP, according to Bovera et al. (2001), as well as in energy by its wealth in starch (Crepon et al., 2010), 50-60 % (Larralde and Martinez, 1991); 42.3 % DM (Saving and al., 2004); 41.1 and 47.5 % DM (Hood-Neifer, 2012). This involves at least a stabilization of the performances, even if not an improvement.

The evolution curve of the ADG during the finishing phase of fattening (figure 1), reveals three periods which are almost equal in time, and the curve of the ADG follows the same allure for the various diets. The first period shows ADG in the ascending phase, and then they show certain stability in the second period. These two periods are the result of returning on to a high feeding level brought up after a certain period of feeding limitation. So this situation allows an improvement of the ADG due to the compensatory growth (Hoch et al., 2003). Moreover, certain bull-calves achieved more than 1.3 kg / day. During the third period (downward phase) the ADG decreases and reaches values even lower than the ADG of departure, showing that animals achieved their terminal phase of fattening and their growth is slows down.

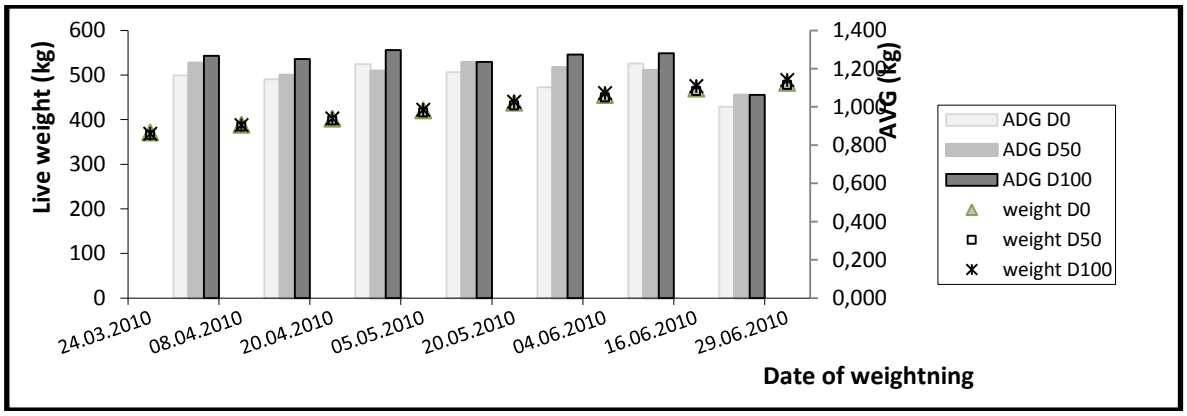

Figure 1: Evolution of live Weight (kg) and ADG (kg) depending on type of diets.

Conclusion

This trial shows that partial or total substitution of soybean by faba bean, doesn't affect the performance of bulls, even it leads to significant better performances marked specifically by a slight decrease in the consumption index and the elevation of Average Daily Gain. These parameters have a good economical interest for bulls' fattener, by reducing the feeding cost and then the cost of 1 kg of meat. Furthermore, the use of faba bean has reduced the feeding cost, particularly when the faba bean is autocultivated by fatteners and not bought from market. Even if the faba bean is bought from market, it leads to producing a concentrate cheaper than the ordinary one composed only of soybean meal.

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EFFECTS OF THE FEEDING LEVELAND OF THE SUBSTITUTION OF THE BARLEY BY THE OAT ON PERFORMANCES OF REPRODUCTION OF RAM

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Abstract

Nine 3-year-old male West Fine-tailedlocal sheep were used to evaluate the effects of the level of the extract as well as the substitution of the barley by the oat on performances of reproduction of Ram. Each animal received, during a first period of the experience, 1.5 kg of oat hay and 400 g of concentrate (A) to basis of barley (80%), of the soybean meal (17.5%) and of mineral and vitamin supplement (2%). Then, animals were divided in two homogeneous groups according to age, the live body weight and the volume of ejaculation. Rams of the two groups continued to receive the same ration of basis complemented with 700 g of the same concentrate to basis of barley (B) and 700 g of the concentrate in which the oat replaced the barley (C). The live body weight of animals increases with the level of the extract and the substitution (61.5; 61.8et 60 kg respectively for C, B and A groups) without any difference among treatments. Testicular size was greater ($P < 0.05$) for C than for B all two superior to A groups. The two applied food treatments, an improvement of ejaculate volume (0.5; 0.7 and 0.9 ml respectively for A, B and C), of the number of spermatozoa ($0.6*10^9 \pm 0.3$; $0.9*10^9 \pm 0.2$ and $1.1*10^9 \pm 0.4$spz respectively for A, B and C) but not of the concentration. The increase of the level of the extract and the utilization of the oat improve as meaningfully ($P = 0.0001$) mass motility (4±0.5; 4.2±0.4 and 4.6±0.2 respectively for A, B, C) and individual (3.6±0.5; 3.84±0.4 and 4.43±0.3 respectively for A, B, C). The diet doesn't affect the dead spermatozoa and the total anomalies.

Keywords: *Rams, food Level, Oat, substitution, Reproduction.*

Introduction

Sheep rearing plays an important role for the sustainability of environmental and farming systems in Tunisia. Sheep meat is very important in various regions of the country. It covers subsistence farming but also supplies the market with diverse reasons such as demographic, religious and socio-economic factors. Sheep meat production has increased considerably, from 45,200 to 50 000 tons between 2004 and 2011 (Givlait, 2011) West Fine-tailed Sheep specialized in meat production represents 35% of Tunisian sheep flock, adapted to extensive and semi-intensive livestock system. Coverage of the demand in sheep meat requires the intensification of production this can be done by increasing the weight of lambs and the decrease in age at slaughter or /and increase the number of products lambs. Accelerating the pace lambing (3 lambing / 2 years) to increase the number of products lams,this requires optimal sexual activity for both sexes. However, there are many studies on sheep reproduction but there are a few researches regarding the effect of diet on semen of ram. The purpose of this present study was to focus on the effects of the level of the extract and of the substitution of the barley by the oat on performances of reproduction of ram.

Materials and methods

The experiment was carried out in Tunisia at the experimental station of the Higher School of Agriculture of Kef, Tunisia (ESAK). The station has a semi-arid climate and is located 8 km south of the town of Kef 35°7 ’ N latitude. Nine adultQueue Fine de l’Ouest rams with an average initial body weight of 59.1±10.89 kg.The rams were housed in individual pens where fresh water was freely available.Experiment was conducted between February and June 2008. During the first trial period(February to April) each animal of group A received 1.5 kg of oat hay/ram/day and daily supplemented with 0.400 kg of concentrate containing 80% barley, 17.5% soybean and 2.5% a mineral and vitamin supplement. After in the second period, rams were allocated to two groups balanced for age, live weight and ejaculate volume. Rams in B group received the same diet as group A but the concentrate was increased to 0.700 Kg. Rams in C group were fed a diet composed of 1.5 kg of oat hay/ram/day and 0.700 Kg of concentrate containing 80% oat, 17.5% soy bean and 2.5% a mineral and vitamin supplement. Measurements of live weight and testicular size were performed every week, however volumes of sperm and semen traits were performed 4 times per week (Tuesday: 2 ejaculates, Friday 2 ejaculates) using an artificial vagina. All semen parameters were measured by the same person throughout the study. Immediately, concentration and sperm motility (0-5) were assessed subjectively using a phase-contrast microscope on a warm water bath (37°C)(Soyluet al., 2007).For the sperm morphology assessment, the percentage of abnormal sperm (detached heads, abnormal mid-pieces, or tail defects) was recorded by counting a total of 150 spermatozoa under phase contrast microscopy (×1000)

Results and discussion

Composition of diets

The chemical composition of different diets is documented in Table 1

Table 1. Chemical composition (%, DM basis)

Nutrient	**Concentrate**		**hay**
	berly	**Oat**	
Dry matter	87.7	88.1	87.1
Organicmatter	96.5	96.8	91.2
Crude fibre	8.9	12.4	45.3
Crudeprotein	12.6	14.3	4.5

The results of this analysis show that the hay is low in CP (4.5%) and rich in crude fibre (45.3%) confirming the results reported by several studies (Mahouachi and Khaldi, 1987).The CP and crude fibre concentrations in the diets containing oat were greater than diet containing berly.

Body weight

Fig.1 shows that the body weight of animals increased slightly in the first period from 59.1 ± 10.2 kg (W1) to 60.84 ± 9.9kg (W9). During the second period of the experiment, the body weight was increased. Three weeks after the change of diet, the live weight of rams is 61.82 ± 9.6 kg and 62.2 ± 9.9 kg for respectively B and C. At the 14th week the body weight decreased is probably due to shearing rams.

Fig1. Effect of the level and nature of the energy source in the concentrate on body weight changes.

As shown in Table 2, there was a tendency for BW to increase in C group in comparison to B group. However, this difference was not statistically significant between this to groups. But a statistical difference was observed between the group A and the two groups. So the level of energy in the diet affects the BW. However,BW was not affected by the nature of the energy in diet.

Table2. Effect of diets on body weight (BW), Mean (± SEM)

Group	BW
A	$60 \pm 10{,}2^{b}$
B	$61{,}8 \pm 10^{a}$
C	$61{,}5 \pm 9{,}9^{a}$

Means with different superscripts (a, b) within each column are significantly different (P<0.05).

Quantitative semen parameters

Two types of examination were performed: macroscopical and microscopical examination. The macroscopical examination included the measurement of the ejaculate volume, the color and smell of semen were assessed. (Table 3)

Table 3. Macroscopic examination of semen

	Semen volume (ml)	**Color**	**Consistency**	**Density**	**Smell**
A	0,5	White	creamy	dense	normal
B	0,7	White	creamy	dense	normal
C	0,9	White	creamy	dense	normal

As shown in Table 4, the testicular size increased for the group C (TSL: 4.8 ± 1.12 cm and TSR: 4.6±1.1 cm)compared with the group B (TSL: 4.1 ±0.47 cm and TSR: 3.9±0.52cm). There was a tendency for ejaculate volume to increase in C group in comparison to A and B groups (0.5±0.1; 0.7±0.2; 0.9±0.4 ml for respectively A, B and C rams).There were significant (P<0.05) differences between diets in both testicular size, ejaculate volume, sperm concentration and spermatozoa number (Mahouachi et al., 2011). These findings agree with those reported by Fernàndez et al. (2004). Although these results are inconsistent with those obtained byLassoued et al.(2013), Bielli et al. (1999) and Lindsay et al. (1984), who found no significant effect between improved pasture or high dietary protein on quantitative semen parameters.The nature of the energy in diet affects the testicular size but not the body weight. These results led to the conclusion that testicular size is more sensitive to changes in diet than body weight indicating a preferential allocation of nutrients to the reproductive system.

Table 4. Effect of regimes on testicular size (TS), ejaculate volume (EV), sperm concentration (SC) and spermatozoa number, mean (± SEM)

Experimental diets	TS (cm)		EV (ml)	SC($\times 10^9$/ml)	Spermatozoa Number
	Left	Right			
A	3.8 ± 0.86^a	3.5 ± 0.81^a	0.5 ± 0.1^a	$1.2\ 10^9\pm0.1^a$	$0.6*10^9\pm0.3^c$
B	4.1 ± 0.47^b	3.9 ± 0.52^b	0.7 ± 0.2^b	$1.2\ 10^9\pm0.08^a$	$0,9*10^9\pm0.2^b$
C	4.8 ± 1.12^c	4.6 ± 1.1^c	0.9 ± 0.4^c	$1.3\ 10^9\pm0.1^a$	$1.1*10^9\pm0.4^a$

Means with different superscripts (a, b, c) within each column are significantly different (P<0.05).

Qualitative semen parameters

Rams that received oat had the highest mass motility (4.6±0.2 for C, 4.2±0.4 for B and 4±0.5 for A) during the experiment (P < 0.05).Diet with oat affects more the mass motility as diet with barley. The highest effect on ram sperm individual motility was observed with the diet of oat(3.6±0.5; 3.84±0.4; 4.43±0.3 for respectively A, B and C rams). However, the results (Table 5) indicated that the diet significantly affected the individual motility (P<0.01).Results of our work confirm that oat affects sperm motility parameters. Oat is more energetic than barley. Mahouachi et al. (2011)reported similar findings.

Table 5. Effect of regimes on Mass Motility (MM) and Individual Motility (IM), Mean (± SEM)

Experimentaldiets	MM	MI
A	$4\pm0,5^a$	3.6 ± 0.5^a
B	4.2 ± 0.4^b	3.84 ± 0.4^b
C	4.6 ± 0.2^c	4.43 ± 0.3^c

Means with different superscripts (a, b, c) within each column are significantly different (P<0.05).

The data on dead spermatozoa and abnormal spermatozoa are presented in Table 6.Dead sperm was higher in A (15.25%) compared with B (13.59%) and C (9.32%).The percentage of abnormal spermatozoa decreased with the level of the extract and of the substitution of the barley by the oat. These data indicated that, the dead spermatozoa and the abnormal spermatozoa were not significantly affected (p<0.01) with the diet.Martin andWalkden-Brown (1995) and Zahid et al. (2003) observed that diet did not affect the semen quality.

Table 6. The percentage of dead sperm and total abnormality of ram spermatozoa

	% Dead spermatozoa	Tails		% abnormalhead	% detached head
		bent	coiled		
A	15.25	16.36	14.51	0.51	2.21
B	13.59	15.99	11.79	0.99	2.39
C	9.32	12.16	12.91	0.16	2.58

Conclusion

increase of feeding level and of the substitution of the barley by the oat improves the quantitative and qualitative characteristics of sperm of the ram of local breed.

In conclusion, these results suggest that the increase of feeding level and of the substitution of the barley by the oat improves the quantitative and qualitative characteristics of sperm of the ram of local breed. Nevertheless, the diet doesn't affect the dead spermatozoa and the total anomalies.

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THE UTILIZATION OF MOBILE LAB FOR MEASURING GAS AND PARTICULATE MATTER EMISSIONS OF ANIMAL BARNS

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Abstract

Production and emissions of ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) gases and particle matter (PM) are a result of animal husbandry processes. These pollutants can have harmful effects on growth performance of animals and create uncomfortable conditions for people working in the production facilities. In addition, some of the gases from these facilities can impact the atmospheric environment due to their greenhouse effects. Consequently, determination and mitigation of harmful gases and PM emissions are of importance to both protecting the environment and animal and human health. Although a number of approaches are available to quantify these gases and PM, mobile lab system offers many unique advantages. In this paper, we describe the working principles and measurement system components of a Mobile Lab. Examples of monitoring the gases and PM in commercial animal production facilities with the system are also provided.

Keywords: *Mobile Lab, Greenhouse gases, Gas measuring system*

Introduction

Harmful gases and particle matter in animal feeding operations create uncomfortable situation affecting animals and people working in the facility. Moreover the gases in the barn have been one of the major environmental concerns because they are related to global climate change (Ni et al., 2008). These harmful gases that affect air quality are ammonia, carbon dioxide, methane, and nitrous oxide. Most certainly livestock production is blamed to bear the bulk of gaseous emissions in farming that impacts the environment (Schiefler, 2013). Both quantification and mitigation of aerial emissions from animal feeding operations continue to draw attention in animal industry, academia, and environmental regulatory agencies (Xin et al., 2009).

Mobile air emissions monitoring units (MAEMUs) are capable of precise and real-time measurements of concentrations and emissions and are being used typically for continuous and intensive monitoring of emissions from mechanically ventilated animal facilities (Ricardo et al., 2009). The Mobile Laboratory (Mobile Lab) Measurement System that uses photoacoustic multi-gas analyzers can be moved to any desired location and used on a multiple-site basis. Both longevity and availability of Mobile Lab for different agricultural structure types make this system very attractive. Photoacoustic multi-gas analyzers have been widely used by European scientists and U.S. scientists in air emission studies (Fergusun et al., 1998ab; Fenyvesi, et al., 2001; Nicks et al., 2003; Guarino et al., 2003; Li, 2006).

The objectives of this study were to: a) introduce an MAEMU for measuring harmful gases and PM of animal barns that was designed and developed at Iowa State University (ISU); and b) provide examples of the MAEMU application.

Materials and methods

Technical information about of MAEMU

The ISU MAEMU consists of the following components: 1) gas sampling system (GSS); 2) gas analyzer; 3) environmental sensors; 4) hosting computer; 5) data acquisition system; and 6) other peripheral devices and equipment. The measurements of gaseous and PM concentrations and emissions carried out by the system allow for periodic and simultaneous monitoring of a number of buildings. The gaseous concentrations are measured using the instrument called photoacoustic multi-gas analyzer (INNOVA Model 1412, Ballerup, Denmark), and PM concentrations are measured with tapered element oscillating microbalance (TEOM, Model 1400a) (Moody et al., 2008; Stinn, et al., 2013)

General information about the INNOVA-1412 photoacoustic multi-gas monitor

There are several areas of application for the INNOVA-1412 photoacoustic multi-gas monitor: 1) monitoring of greenhouse gases (GHGs) emissions that result from agricultural production processes; 2) measurements of indoor air quality; and 3) determination of building ventilation rate using a tracer gas. The photoacoustic multi-gas monitoring system INNOVA-1412 has proven to be both reliable and stable gas-monitoring system, providing highly accurate measurements (Figure 3). The INNOVA-1412 engages the photoacoustic infrared detection method for its measurement system. It has a capability of providing measurements for various levels of almost any gas that can absorb the infrared light. The optical filters are used to provide for gas selectivity where the INNOVA-1412 has up to five of these filters installed (Moody et al., 2008; Anonymous, 2014a). Figure 1 describes both the internal structure and the interconnection of the device. The interior pump designed to draw the air from the sampling point into the air filter, so it can flush out the "old" air in the measurement system, replaces it with the "new" air sample. The closure of both inlet and outlet valves allow for the "new" air sample to get tightly sealed in the analysis cell. The infrared light source emanates the light which reverberates from the mirror thereafter passing through the mechanical chopper. The chopper forces the light to pulsate and to go through one of the optical filters fixed on the filter wheel (Moody et al., 2008; Anonymous, 2014a).

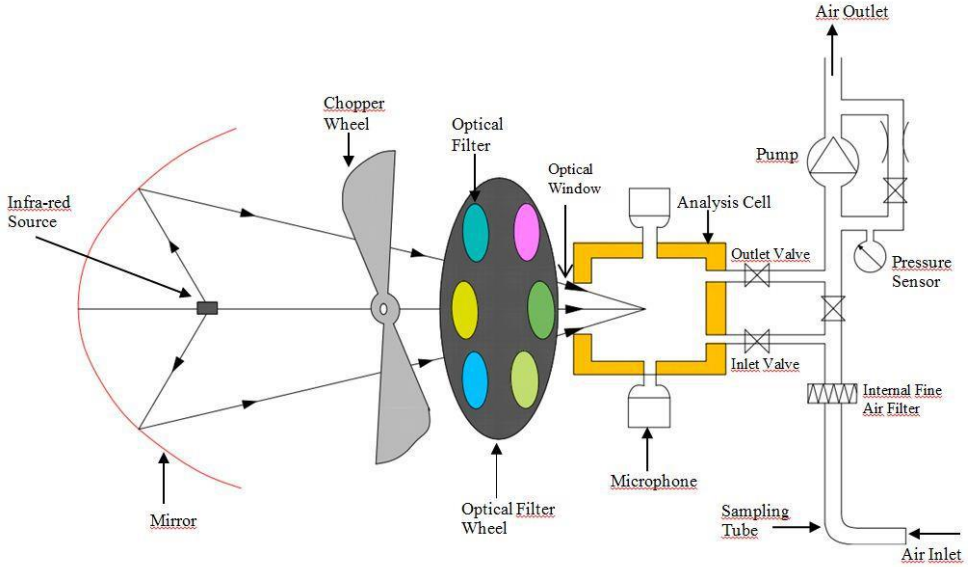

Figure 1. The schematic diagram and the internal structure of INNOVA-1412

General information on the “1400a” series monitor

Air pollution monitoring networks have widely adopted the ambient particulate monitor TEOM (series “1400a”) to be their first choice as it comes to continuous measuring of particulate mass concentrations. The “1400a” series monitor consists of two basic parts: 1) the sensor unit which has the sample inlet and the mass transducer, and 2) the control unit which

has the operator terminal and the box of control electronics. The two parts are connected together using the cable assembly. The “1400a” monitor is the instrument designed on the true gravimetry principle. The ambient air is drawn through the filter with a constant flow rate while the filter itself is being continuously weighed. The mass concentrations are calculated on a near real-time basis (Moody et al., 2008, Anonymous, 2014b).

The gas sampling system

Measurements of air emissions from the animal barn are carried out by the positive pressure gas sampling system that is a part of the MAEMU. The system uses individual pumps to draw sample air continuously from all the locations. If the sample air is not analyzed, it is being bypassed. A four-point gas sampling system is illustrated in Figure 2. The air delivered from the sampling locations via the solenoid valves and into the manifold M1 with the assistance of vacuum pumps P1-P4, then transported to another manifold M2 that is connected to the INNOVA-1412. All wetted parts of the sampling system use either Teflon or Teflon-coating (including the pumps, the solenoid valves, the manifold, and the air tubing). In order to allow measurements of the gas concentrations when automatic gas sampling system is used in each location, both data acquisition system (DAQ) and control unit are used to control the four pairs of the two-way solenoid valves S1-S8 as the process takes place. In order to draw air continuously from each of the sampling locations and avoid potential cross-contamination among the sample locations, individual supply pumps with 16 L/min delivering capacities are used (Moody et al., 2008; Burns et al., 2009).

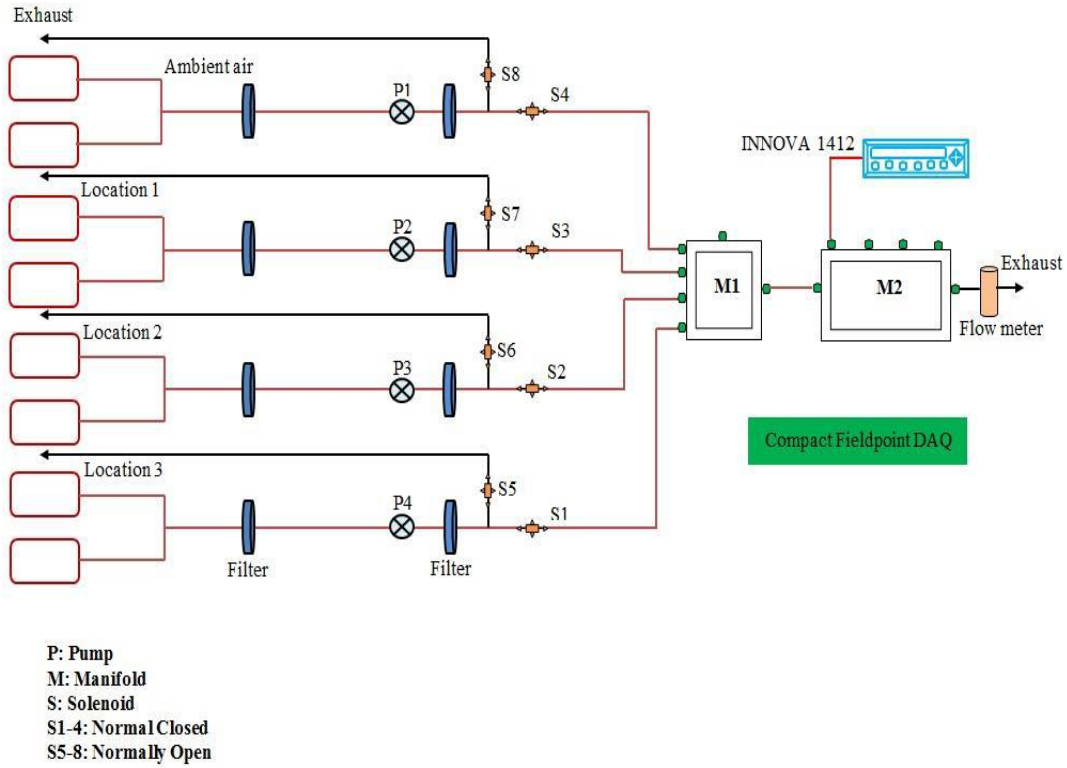

Figure 2. Schematic representation of the positive-pressure gas sampling system used in the MAEMU for measurement of animal barn air emissions.

Sampling of environment conditions

Indoor and ambient relative humidity (RH) is monitored with the electronic RH transmitters, while air temperatures at the air sampling locations and outdoors are monitored with the type T thermocouples. The Temperature and RH sensors are connected to the Compact Field Point modules (National Instruments, Austin, TX) and the PC-based DAQ that is run by LabVIEW 7 program. The program developed by the ISU research team allows for automated sampling location control and display of the real-time data (Moody et al., 2008; Burns et al., 2009).

Results and discussion

The MAEMUs have been successfully used for AFO air emissions research at Iowa State University. Gaseous concentrations (NH_3, CO_2, and CH_4) were quasi-continuously monitored using the fast-response stable H_2O-compensated photoacoustic multi-gas analyzer (INNOVA Model 1412, Ballerup, Denmark). PM_{10} and $PM_{2.5}$ concentrations were measured with the TEOM 1400a units. Specifically the following field studies have been conducted using the MAEMU system.

Li et al, (2011a) carried out a study to determine particulate matter concentration and emissions at a high-rise layer house in Iowa. Two TEOM PM monitors were used in this study to evaluate particular matter concentration. One TEOM monitor measured PM10 concentration, while the other measured PM 2.5 concentration. They determined particular matter concentration ranged from 90 to 1387 μg m^{-3} and from 11 to 168 mg m^{-3} for PM10 and PM2.5 respectively.

Li et al (2011b) have been continuously monitored NH3, PM10, and PM2.5 emissions in turkey barn by using the state-of-the-art mobile air emissions monitoring units (MAEMUs). Average daily NH3, PM10, and PM2.5 concentrations in the tom turkey barn were 8.6 ppm, 1104 g m^{-3}, and 143 g m^{-3}, respectively.

Li et al (2012) have applied three different diets to laying-hen (EcoCall, DGGS and Control diet) to reduce ammonia levels. Also, The MAEMU used to continuously collect data on NH_3,-H_2S, and carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emissions from the three laying-hen houses. The EcoCal and DDGS diets led to an overall NH_3 emissions reduction of 39.2% and 14.3%, respectively, relative to the control diet.

Hayes et al (2013) were determined daily concentrations and emission rates of ammonia; the greenhouse gases and particulate matter in aviary houses. They found daily indoor NH_3, CO_2, CH_4, PM10, and PM2.5 concentrations as 8.7 ppm, 1,636 ppm, 10.0 ppm, 2.3 mg m^{-3}, and 0.25 mg m^{-3}, respectively. A fast-response, high-precision infrared (IR) photoacoustic multigas analyzer and real-time tapered element oscillating microbalances (TEOM) equipped were used on determining daily concentration and emission of gases and particular matter respectively.

Stinn et al (2013) used the MAEMU to continuously collect data on gaseous concentrations, thermal conditions, and operational status of the ventilation fans from the barns and farrowing rooms. The NH_3, CO_2, CH_4, and N_2O concentrations of the breeding/gestation barns found 9.7 ppm, 1536 ppm, 78.3 ppm, and 0.30 ppm respectively. Also, farm-level emission rates of NH_3, CO_2, CH_4, and N_2O gases found 38.5, 8731, 301 and 0.24 g AU^{-1} d^{-1}, respectively.

Zhao et al (2015) used the MAEMU to determine the concentrations of NH_3, CO_2, CH_4, N_2O, and dew-point temperature in three commercial laying hen houses (CC, EC and AV). The highest level of ammonia concentration was found in the AV house.

Shepperd et al (2015) monitored gaseous and particulate matter emissions from three commercial laying hen houses (CC, EC, and AV) and their respective manure storage data of gaseous concentrations. The gas concentrations of NH_3, CO_2, CH_4, NO_2, and dew-point temperature (DP) were measured with a photoacoustic multigas analyzer, for particulate matter concentrations tapered element oscillating microbalances was used. . The highest ammonia and particular matter emission were found in the AV laying hen house.

Acevedo et al. (2009) reported that the MAEMUs are capable of real-time measurements of emissions precisely and they are being used typically for continuous monitoring of emissions from mechanically ventilated animal facilities. Xin et al. (2009) measured GHG and PM emissions using MAEMU from two commercial broiler houses in western Kentucky, USA. Schiefler (2013) used the Innova model 1412 multi-gas analyzer to measure GHG and ammonia emissions from dairy barns.

Accurate ventilation rate data are essential to calculate gas emission measurements from any animal barn. To be able to calculate reliable and real emission values, fans should be controlled when they in service as well as performance values should be considered. Because, emission rates are obtained as the product of two measurements: gas concentration difference between discharge air and ambient air, and ventilation rate (Gates et al., 2004; Li et al; 2009).

Conclusions

Mobile Lab measurement system can be used in different agricultural structures and multiple barns to evaluate gas emissions simultaneously. Although the initial investment costs are rather high, considering environmental impacts as well as employees' health, these systems offer numerous advantages. Moreover, the Mobile Lab Measurement System can be moved to any desired location and used on a recurring basis. Both exploitation period and adaptability of Mobile Lab for different types of agricultural structures make that system quite attractive for studies of gas emissions.

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DETERMINATION OF HEATING AND COOLING DAY DATA FOR TOMATO PLANT: ADANA CASE

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Abstract

Greenhouse cultivation is carried out in two periods during spring and autumn at the Adana region for the tomato plant. At the end of these two periods; the number of heating days was determined to be greater for autumn cultivation, whereas the number of cooling days was determined to be greater for spring cultivation. It is possible to gain some knowledge beforehand regarding the energy consumption of the agricultural structure to be built in the region via the heating and cooling degree days method. When the number of ideal days acquired using the long term meteorological data of the study area is examined, it was determined that the ideal temperature values were obtained for only one day during harvesting period in spring cultivation and for only one day during ripening in autumn cultivation. It was concluded as a result of the study that heating or cooling system will be needed in the greenhouse environment for spring and autumn cultivation of tomato plant.

Keywords: *Adana, Cooling degree day, Heating degree day, Greenhouses, Tomato plant*

Introduction

Greenhouses in which cultivation is carried out by keeping the atmospheric conditions under control are agricultural structures in which plant cultivation can be carried out all year round. The objective in greenhouse cultivation is to attain the highest economic efficiency for cases when the optimum environmental conditions and cultivation environment required for the growth and development of the plants are provided in case external environmental conditions are not suited for cultivation. In other words; greenhouses are controlled cultivation environments which provide the proper climate conditions at an optimum level during the growth period of the plants. It is possible to provide the temperature values suited for the plants during cold days and nights by heating the greenhouses and similarly by cooling the greenhouses during hot days and nights (Yuksel and Yuksel, 2012).

The number of businesses that carry out greenhouse cultivation at the city of Adana and its districts is lower in comparison with Mersin and Antalya. Farm cultivation is carried out more at Adana where cultivation in low tunnels is also carried out. Greenhouses with glass and plastic covers can be found more at the coastal areas of Karatas and Yumurtalik districts. In our country, the city of Adana ranks second after Antalya in terms of greenhouse (low tunnel) area size (TUIK, 2015).

The hardness of a climate can be examined sensitively in units of degree-day. Different climate conditions in different regions may have significant effects on heating or cooling energy demand. Degree-day numbers are used all over the world especially for heating, cooling and cultivation to determine degree-day regions that are different than geographical regions (Lstiburek, 2001; Sahal, 2006; Bulut et al., 2007).

The objective of this study was to determine the number of heating and cooling days using the degree-day method and long term meteorological data for the Adana region where spring and autumn cultivation is carried out for tomato plant.

Materials and Methods

The daily temperature data spanning a period of 55 years for the city of Adana was selected as material for this study. Greenhouse cultivation is carried out in two seasons in the city of Adana: spring and autumn. Therefore, long term temperature data were handled separately for periods when spring and autumn cultivation is carried out. The temperature values suggested for the tomato plant during the cultivation period were evaluated according to Seniz (1992).

Degree-Day (DD) Method**:** In general, a degree-day value is stated as a temperature that is added to the ambient temperature. These values are determined based on the quantity and duration of temperature and according to whether the selected equilibrium temperature is lower or higher than the ambient temperature (Matzarakis and Balafoutis, 2004).

Heating Degree-Day (HDD) Method**:** The temperature difference when daily average external temperature decreases below the equilibrium temperature selected for the greenhouse is expressed as a Heating Degree-Day (HDD) (Satman and Yalcinkaya, 1999).

$$\text{For } (T_O < T_b),\ HDD = \sum_{i=1}^{n} (T_b - T_O) \quad (1)$$

Here; HDD is the cumulative sum of the heating degree-days for n days, n is the total number of days in the period, T_b is the greenhouse equilibrium temperature suggested for the tomato plant and T_o is the average external temperature (Gultekin, 1995; Yucel et al., 2014).

Cooling Degree-Day (CDD) Method**:** Cooling degree-day (CDD) values are mathematically defined as the difference between the external temperature value and the equilibrium valueand are defined as the equilibrium temperatures above the external temperature values (Krese et al., 2012; Yucel et al., 2014).

$$\text{For } (T_O > T_b),\ CDD = \sum_{I}^{n} (T_O - T_b) \quad (2)$$

Here; CDD is the cumulative sum of the cooling degree-days for n days, n is the total number of days in the period, T_b is the greenhouse equilibrium temperature suggested for the tomato plant according to Seniz (1992) and T_o is the average external temperature.

Heating Degree-Day Value (HDDV) Method: Heating degree-day values are defined as the difference between the equilibrium temperature and air temperature for each day. Many methods and approaches have been given in literature for the calculation of degree day values (Baskerville and Emin, 1969; Floyd and Braddock, 1984; Yang et al., 1995; McMaster and Wilhelm, 1997; Martinaitis, 1998, Matzarakis and Balafoutis, 2004). HDDVs have been calculated using the equation 3 given below (Buyukalaca et al., 2001; Yucel et al., 2014).

$$HDDV = \sum_{i=1}^{n} HDD \quad (3)$$

Here; n is the total number of days which were HDD during the selected period

Cooling Degree-Day Value (CDDV) Method**:** In this method developed similar to HDDV values, the number of days during the cultivation period in which the average external temperature values for the Cooling Degree-Days were higher than the equilibrium temperature values suggested for the examined tomato plants have been calculated via equation 4 given below (Buyukalaca et al., 2001; Yucel et al., 2014).

$$CDDV = \sum_{i=1}^{n} CDD \quad (4)$$

Here; n is the total number of days in the selected period which were CDD.

Results and discussion

Daily average temperature values between 01.01.1960 and 31.12.2014 along with the equilibrium temperature values suggested for the tomato plant were used to calculate the

number of heating and cooling days for each cultivation period. The temperature data between the start and end dates for both periods were examined according to the cultivation periods of the tomato plant.

Spring cultivation

Spring cultivation in the region starts in the first week of January and the harvest period continues until the end of June. The plantation period of the seedlings covers a period of one week. When the temperature values on these dates are examined, the number of heating days in the greenhouse for tomato plant was determined as (7 days) for both the suggested temperature values (12-22 ^{o}C) and the average temperature value (17^{o}C) (Table1).

Table 1. Heating and cooling day values during the sowing and plantation period of spring cultivation

	Desired temperature in the greenhouse (^{o}C)	Heating number day	Cooling number day	That should not be heating and cooling number of days	Sowing and plantation period (day)
Mean	12	7	0	0	7
	13	7	0	0	7
	14	7	0	0	7
	15	7	0	0	7
	16	7	0	0	7
	17	7	0	0	7
	18	7	0	0	7
	19	7	0	0	7
	20	7	0	0	7
	21	7	0	0	7
	22	7	0	0	7

Numbers of flowering, pollination and fertilization period heating and cooling days were given in Table 2. It was determined that this is a period of two weeks and all of the examined days were determined as number of heating days.

Table 2. Heating and cooling day values during the flowering and pollination period of spring cultivation

	Desired temperature in the greenhouse (^{o}C)	Heating number day	Cooling number day	That should not be heating and cooling number of days	Flowering and pollination period (day)
Mean	18	14	0	0	14
	19	14	0	0	14
	20	14	0	0	14

When the next stage of spring cultivation which is the fruit set period is examined, it was observed that this period covers a period of one month (Table 3). It was determined that the number of cooling days increased with increasing ambient temperature values during this period. Still, it was determined that the number of heating days increased for values above 19 ^{o}C during this period when the temperature demand is high.

Table 3. Heating and cooling day values during the fruit ripening period of spring cultivation

	Desired temperature in the greenhouse (^{o}C)	Heating number day	Cooling number day	That should not be heating and cooling number of days	Ripening period (day)
Mean	15	8	22	0	30
	16	10	20	0	30
	17	11	19	0	30
	18	22	8	0	30
	19	25	5	0	30
	20	30	0	0	30
	21	30	0	0	30
	22	30	0	0	30
	23	30	0	0	30
	24	30	0	0	30
	25	30	0	0	30

When the data for the final period of spring cultivation, harvesting period are examined, it was observed that the period covers a period of 2 months (Table 4). It was determined that the number of cooling days during this time period increased along with the increase of the temperature demand of tomato plant. It was determined that the number of cooling days was 6 at the average temperature value.

Table 4. Heating and cooling day values during the harvesting period of spring cultivation

	Desired temperature in the greenhouse (^{o}C)	Heating number day	Cooling number day	That should not be heating and cooling number of days	Harvesting period (day)
Mean	18	0	60	0	60
	19	0	60	0	60
	20	3	57	0	60
	21	11	49	0	60
	22	17	43	0	60
	23	22	38	0	60
	24	28	32	0	60
	25	38	22	0	60
	26	49	11	0	60
	26,5	53	6	1	60
	27	56	4	0	60
	28	60	0	0	60
	29	60	0	0	60
	30	60	0	0	60
	31	60	0	0	60
	32	60	0	0	60
	33	60	0	0	60
	34	60	0	0	60
	35	60	0	0	60

Autumn Cultivation

Autumn cultivation starts on the third week of August and continues until the 3rd week of January. When the temperature values during this period are examined, it was observed that the number of cooling days was 7 for the 1 week period at both the suggested temperature values (12-22 ^{o}C) and the average temperature value (17 ^{o}C) during the plantation period (Table5).

Table 5. Heating and cooling day values during the sowing and plantation period of autumn cultivation

	Desired temperature in the greenhouse (^{o}C)	Heating number day	Cooling number day	That should not be heating and cooling number of days	Sowing and plantation period (day)
Mean	12	0	7	0	7
	13	0	7	0	7
	14	0	7	0	7
	15	0	7	0	7
	16	0	7	0	7
	17	0	7	0	7
	18	0	7	0	7
	19	0	7	0	7
	20	0	7	0	7
	21	0	7	0	7
	22	0	7	0	7

All 14 days in autumn cultivation period were determined as cooling days contrary to spring cultivation when the flowering, pollination and fertilization periods are examined (Table 6).

Table 6. Heating and cooling day values during the flowering and pollination period of autumn cultivation

	Desired temperature in the greenhouse (^{o}C)	Heating number day	Cooling number day	That should not be heating and cooling number of days	Flowering and pollination period (day)
Mean	18	0	14	0	14
	19	0	14	0	14
	20	0	14	0	14

It was determined during the study that the fruit set period covers a period of 30 days during autumn cultivation. Cooling days ended and heating days started as cultivation advanced and temperatures exceeded 23 ^{o}C (Table7).

Table 7. Heating and cooling day values during the fruit ripening period of autumn cultivation

	Desired temperature in the greenhouse (^{o}C)	Heating number day	Cooling number day	That should not be heating and cooling number of days	Ripening period (day)
Mean	15	0	30	0	30
	16	2	28	0	30
	17	8	22	0	30
	18	12	18	0	30
	19	17	13	0	30
	20	18	11	1	30
	21	25	5	0	30
	22	28	2	0	30
	23	30	0	0	30
	24	30	0	0	30
	25	30	0	0	30

When Table 8 is examined regarding the harvesting period in autumn cultivation, it can be observed that this period covers 60 days. It was determined that all days in the period were heating days due to the decrease in temperature.

Table 8. Heating and cooling day values during the harvesting period of autumn cultivation

	Desired temperature in the greenhouse (^{o}C)	Heating number day	Cooling number day	That should not be heating and cooling number of days	Harvesting period (day)
Mean	18	60	0	0	60
	19	60	0	0	60
	20	60	0	0	60
	21	60	0	0	60
	22	60	0	0	60
	23	60	0	0	60
	24	60	0	0	60
	25	60	0	0	60
	26	60	0	0	60
	26,5	60	0	0	60
	27	60	0	0	60
	28	60	0	0	60
	29	60	0	0	60
	30	60	0	0	60
	31	60	0	0	60
	32	60	0	0	60
	33	60	0	0	60
	34	60	0	0	60
	35	60	0	0	60

It was determined at the end of the study that the numbers of heating and cooling days for tomato plant cultivation periods met the ideal temperature values for 1 day during the harvest period in spring cultivation and for one day during the fruit set period in autumn cultivation.
The cultivation dates suggested for the tomato plant during the Spring and Autumn period were evaluated according to Anonymous (2002).

Conclusion

Greenhouse cultivation is carried out in two seasons, spring and autumn, for tomato plant in the Adana region. At the end of these two cultivation periods; it was determined that the number of heating days were greater for autumn cultivation, whereas the number of cooling days were greater for spring cultivation. Knowing the sum of the numbers of heating or cooling days is important for calculating the energy requirement (Ulupınar et al., 2012; Yucel et al., 2014). It was determined as a result of this study that energy requirement related with cooling is greater for spring cultivation and that the energy requirement related with heating is greater for autumn cultivation periods.

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MANAGEMENT OF MANURE FROM LIVESTOCK HOUSING AND ITS ENVIRONMENTAL POTENTIAL IMPACT ON CEYHAN AND SEYHAN RIVER

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Abstract

Manure that is not properly managed and is stored in open stacks outside causes odor pollution, visual pollution and water pollution. Problems caused by animal manure from livestock building were determined and necessary explanations were made about utilization of animal manure. Manure should be either stored under proper conditions or properly managed in order to prevent such problems.

The study material was selected as livestock enterprises with ongoing production close to the coastal regions of Seyhan and Ceyhan Rivers. About 20000 cattle are bred in the cattle breeding enterprises in the region. However, 8046 are bred for commercial purposes. The management of animal manure from livestock depending on the capacity of current animal breeding enterprises was made. It was concluded that animal manure which is the output of animal breeding enterprises will result in environmental pollution, water resource pollution as well as posing a threat to life in general by mixing with water resources such as underground water, rivers etc. In addition, the needed precautions for the purpose waste materials not causing environmental pollution.

Key words: *Livestock, Manure management, Seyhan and Ceyhan River*

Introduction

The characteristics of manures obtained from barns differ significantly among farms. The age of the animal, its gender, species, ration, production method and other factors might be shown as factors that affect the characteristics of manure (Anonymous, 2003). Animal based can act as a good plant nutrient if it is managed properly and is applied in a responsible manner (Harris et.al., 2001; Cayley et.al., 2004). However, excessive use of commercial and animal based manure creates serious pollution in addition to potential sources of pollution (Atilgan et al., 2006).

Animal based manure produced in stock breeding enterprises cannot be used very efficiently due to improper storage conditions. Producers that use animal manure do not know how to use it properly and spread the manure on the field in a random manner. The reasons why the necessary importance is not given to animal based manure are that commercial manure is much easier to use and to find. Turkey has an important place among the countries of the region with regard to agriculture and stock breeding. Since the geography and climate conditions of the country are suited to produce agricultural products, it is one of the few countries that meet their own food product demand via agricultural production (Cayir et al., 2012)

The use of farm manure in a benighted manner that does not comply with the relevant technical methods decreases the effectiveness of farm manure in agricultural production. Significant amount of nutrients are lost because insufficient amount of farm manure is left to wait under inconvenient conditions or due to improper application of manure thus resulting in inefficient usage of farm manure in terms of soil efficiency and agricultural productivity

(Boyaci et al., 2011). Pollution sources at stock breeding enterprises are not point source pollution sources such as industrial and urban pollution sources. The fact that they are spread over wider areas makes it more difficult to know the dimensions of water pollution caused by these sources. Manures and animal based wastes that are identified as non-point pollution sources reach surface waters or ground waters thus disrupting the quality of water resources and rendering them unusable (Ozek, 1994; Ongley, 1996; Cayir et al., 2012).
The objective of this study was to determine the probable negative effects of animal manure at stockbreeding animal barns along the Ceyhan and Seyhan Rivers that continue their agricultural activities on the environment and especially on water resources thus attracting attention to this issue.

Materials and Methods

The study was carried out in 2015 t at the region encompassing the area where the Seyhan and Ceyhan rivers flowing to the Mediterranean are located in. This area is known in short as the Seyhan or Ceyhan Basin. Seyhan River is one of the rivers in Turkey that flows to the Mediterranean. It has a length of 560 km and a basin area of 20.600 km². The Ceyhan river is 509 km long with a basin area of 21.222 km^2 (Anonymous, 2015). Even though stockbreeding activities are carried out extensively around the study area, the livestock establishments in the villages of the region closest to the river were taken into account since it was thought that their environmental effects would be greatest. To this end, 3 cities (Adana, Osmaniye and K.Maras) and 64 villages were selected for the Ceyhan River. Whereas 37 village centers located in the city of Adana were selected for the Seyhan River. According to the information acquired from the Adana Provincial Directorate of Agriculture; the number of cattle reared at agricultural establishments along the Seyhan River is 3550, the number of cattle reared at agricultural establishments along the Ceyhan River is 4496 for a total number of 8046 commercially reared cattle. The locations of the establishments were determined via Google satellite map and relevant marking was made.

Results and Discussion

The manure that is generated at animal establishments can be an additional source of income for the establishment if it is evaluated in a suitable and controlled manner. On the contrary, manure that is not stored properly and that is left out in the open may suddenly become harmful for the environment. The manure at animal establishments has to be stored properly so that such negativities do not occur.
Animal rearing is still a popular agricultural sector at the study area. Some of the wastes that occur as a result of animal rearing activities is used as natural manure in agriculture; whereas the remaining is stored improperly at open warehouses and/or is dumped to the nearest farmland. Thus, total nitrate and total phosphor loads are generated due to animal manure which is evaluated as animal based waste; which means in other words that they become significant pollutants for the Seyhan and Ceyhan rivers.
The nitrogen and phosphor loads that are diffused to the environment when animal based wastes are used as natural manure vary significantly with regard to animal category, type, feeding habits, weights and fertilizing properties. Thus, it is very difficult to determine the unit loads. However, controlled agriculture activities should be carried out first at the villages around the rivers and the settlement areas which are affected from the creeks that feed the rivers in order to minimize the environmental effects due to manure at the Seyhan and Basins. In addition, manure and agricultural pests used in agricultural activities, all kinds of uncontrolled animal rearing activities, leakage waters that occur as a result of uncontrolled storage of manure are factors that should be taken into account seriously in order to determine the future of the basin.

The satellite images of cattle rearing establishments that continue their activities around the Seyhan River are shown in Figure 1.

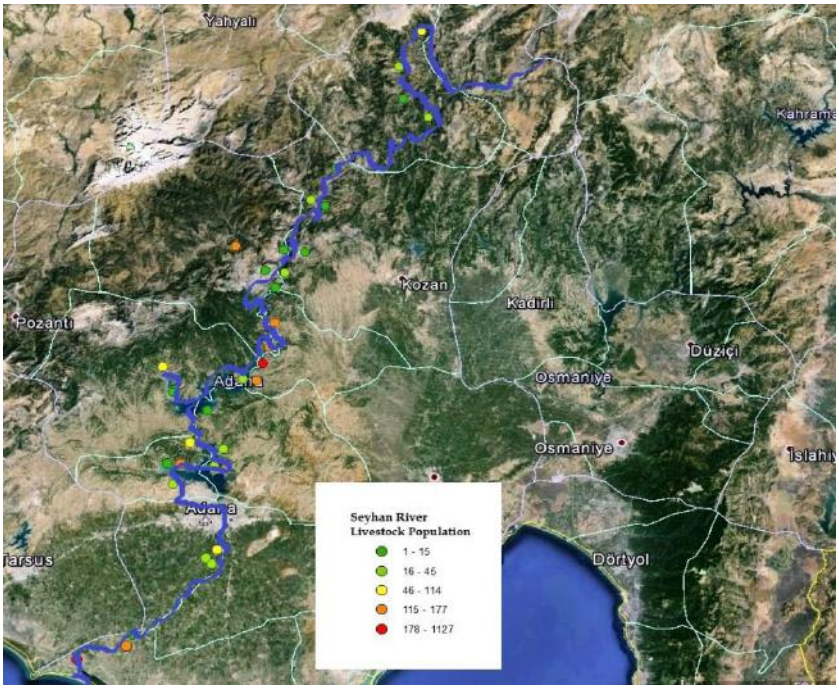

Figure 1. Distribution of animal establishments around the Seyhan River region

When the distribution of the cattle rearing establishments in the region is examined, it was observed that an intensive animal rearing activity is carried out along the river. It is understood from the satellite images of various animal establishments in the region that their locations are close to water sources and that the animal manure that is formed requires a process that is suited to water resources. When the animal manure in these establishments are accumulated in random piles, they create environmental pollution, visual pollution as well as odor pollution while creating potential pollution sources for water sources as well. Because researchers have put forth that the manure piles that are accumulated in the open should be at least 100 meters away from the nearest river, creek and drainage channels. (Mutlu, 1999; Cayley et al., 2004). Care should also be shown for the storage of manure in animal establishments as well as for mixing it with the soil for use in plant cultivation. It should not be forgotten that if the required care is not shown, ground and surface water sources might be polluted in addition to odor and visual pollution, that greenhouse effect might occur due to the gases that are discharged to the atmosphere and that the inorganic nutrient substances in plants (N, P, K) will be washed away with the rains and mix with the lower layers of the soil (Erkan, 2005).

A total of 4496 cattle are reared in the establishments that are currently active around the Ceyhan River. The satellite image of these establishments is shown in Figure 2.

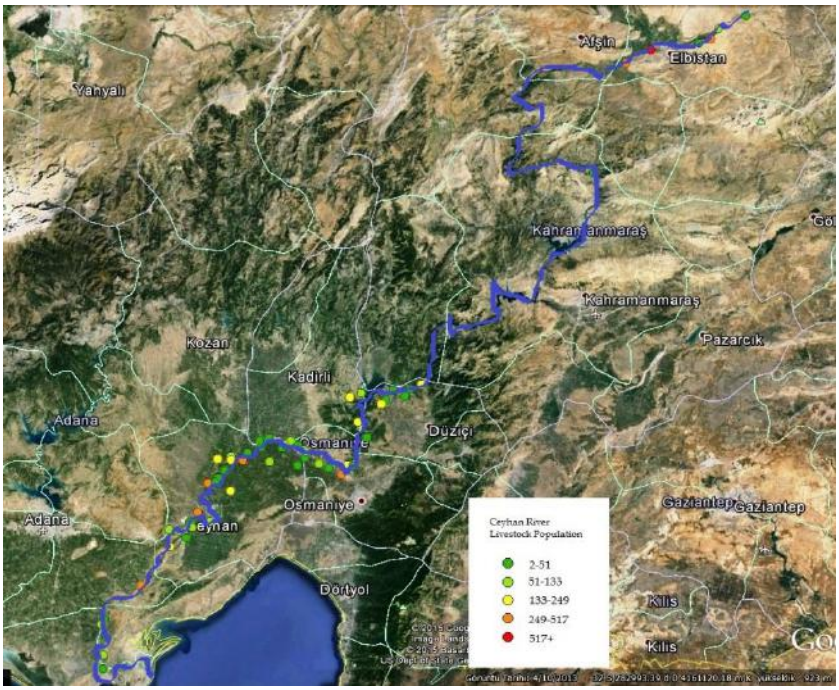

Figure 2. Distribution of the animal rearing establishments in the Ceyhan River region

The mixing of animal wastes to water sources during manure management decreases water quality thus resulting in the death of aquatic living things. The ammonia, pathogens as well as other organic substances with high biological oxygen value result in the contamination of water. The pollution of water might be due to various reasons. The discharge of manure directly to a river, leakage from storage tank or manure storehouses as well as the drainage of wastes to the soil play important parts in the contamination of ground and surface waters (Olgun, 2011).

The main source of river pollution is leakage from sewage as well as from food residues and manure from agricultural areas. The manure and pesticides used in agricultural areas as well as liquid animal manure, other animal residue and wastes cause significant increase in nitrogen and phosphor concentration when mixed with rivers (Gokmen, 2007).

The most important factor that has negative effects on the oxygen balance in the rivers is the consumption of oxygen during the biochemical decomposition of organic substances and nitrous compounds. Even though soluble oxygen is widely found in rivers, a slight decrease in its level may have harmful effects on the living things in rivers. The color and odor of the water changes as a result of river pollution, the plants and animals that live in the water die, microorganisms reproduce, people and animals that make use of these waters get sick or they might be affected dramatically (Gökmen, 2007). We should consume and protect our natural sources in a conscious manner since it takes many years for them to form.

Conclusions

Previous studies put forth that manure storage habits are limited with random storage. This and other similar studies indicate that improperly stored animal manure creates potential pollution sources for our water sources and results in factors that threaten the lives of living things in the environment. However, the precautions that need to be taken have been clearly specified in the relevant literature. It is difficult to state that animal establishments take into account these warnings. Similar results have been obtained from similar studies. It is clear that these studies carried out to protect our environment or to ensure that the manure is properly managed should not be limited only to scientific environments. The precautions to be

taken should be frequently mentioned in the printed press thus becoming accessible for our manufacturers. It was observed that only in this way the results can be effective and that otherwise these efforts will not mean much for the protection of the environment and the water sources.

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EFFECTS OF COLD AND HEAT STRESS ON EGG QUALITY TRAITS OF A NEWLY IMPROVED HYBRID LAYER

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Abstract

ATAK-S is a newly developed hybrid layer. Although the laying performance of this hybrid has been studied and determined, the performance response of the hybrid to different environmental temperature conditions is not known. This study was therefore undertaken to determine the effects of cold and heat stress on egg quality traits. Three hundred and sixty hens were divided into three temperature groups; control (20 ^{0}C), low (12 ^{0}C) and high (32 ^{0}C) in the study. All the hens were held in three tier battery cages in an environmentally controlled research poultry house. The study lasted for 3 weeks. No differences were found between different groups in terms of shape index, albumen height and Haugh unit of the egg quality traits ($P>0.05$). Egg shell breaking strength, egg weight, shell thickness and yolk colour differed significantly between the temperature groups ($P<0.05$) and, the values of these traits decreased under the heat stress conditions, whereas were not affected from the cold stress.

Keywords: *Layer, cold stress, heat stress, egg quality*

Introduction

Since mammals and bird species are warm-blooded animals, they have the ability to sustain their body temperature through heat loss or generation. Normally, the body temperature of hens is around 39.4-40 ^{0}C. They will not be able to continue their normal performance during periods when body temperature is elevated. The thermoneutral zone, which is around 18-23.9 ^{0}C for adult laying hens and 26-27 ^{0}C for adult broilers, is crucial for metabolic and productive activity in hens. Energy is efficiently used for growth, immune system and reproductive activities within thermoneutral zone. On the other hand, hens use a large part of their energy for heat production or loss to control body temperature outside thermoneutral zone. In such cases, the efficiency of feed utilisation decreases (Czaricik and Fairchild, 2008).
Heat stress in laying hens has a negative influence on egg efficiency, egg weight and egg quality traits (Ahvar *et al.,* 1982; Daniel and Bolnave, 1979; Deaton, 1983; Feizi *et al*., 2012; Gill and Ganwar, 1984; Moshaly *et al.*, 2004; Rozenboim *et al.,* 2007). It is reported that there is a variation among lines in terms of tolerance to heat stress and that it is necessary to evaluate genetic differences with regard to the response against heat stress (Felver *et al*., 2012).
Many studies have been carried out to decrease the effects of heat stress on production traits. Dai*et al.* (2009) reported that egg production and feed consumption gradually decreased when the drinking water of hens under heat stress was added with NaCl by 0.2 or 0.4% during the study. The egg production rate decreased in the control group with no supplementation. However the production traits returned to normal levels after the stress period. Another study reported that feed efficiency, egg production and Haugh unit values increased by adding vitaminE to the feeds of hens under stress (Ehran and Bolukbasi, 2011). On the other hand, Yardibi and Turkay (2008) pointed out that adding vitamin E in different dosages to the diet had no positive effect on egg production.

The addition of y-aminobutyric acid (Hui*et al*., 2010), aspirin (ZiaoTing and YouMing, 2002) and betain (Ryu*et al.,* 2002) has been reported to increase or improve performance or egg quality traits such as albumen height, Haugh unit, egg production, feed efficiency, shell weight and shell thickness or egg shell breaking strength during heat stress period.

ATAK-S, which is a newly improved commercial layer, has been developed by crossbreeding of Rhode Island Red and Barred Rock (Durmus *et al.,* 2009). Comparative studies have been undertaken to determine the laying performance of this hybrid. Hens of this hybrid have reached to sexual maturity at an age of 138 days with 1.83 kg of weight on average. Total egg production for 72 weeks has been reported as 312.8 with an average egg weight of 64.1 g. ATAK-S have a high viability rate, which is about 95.9 % (Durmus *et al.,* 2009).

On the other hand, the production response of this hybrid under different temperature conditions (heat or cold stress) has not been studied. The aim of this study was to investigate the production response of ATAK-S hybrid to heat or cold stress conditions.

Material and Methods

This study used 360 ATAK-S commercial laying hens, which were located at the Poultry Research Station. Feeds fed to the hens throughout the study are presented with their chemical compositions in Table 1.

Table 1. Compositions of diet

Ingredients	**0-3 weeks**	**4-10 weeks**	**11-16 weeks**	**17-30 weeks**
	Diet composition, % of the diet			
Maize	56.3	50.7	53.3	56.9
Soybean meal	19.6	14.1	13.0	16.7
Sunflower meal	10.0	10.0	5.1	8.0
Wheat middling	9.7	20.0	-	-
Full fat soybean	-	-	16.0	4.0
Vegetable oil	0.60	1.3	1.4	1.3
Other ingredients and additives	3.8	3.9	11.2	13.1
Nutrients	*Chemical composition*			
Dry matter, min (%)	88	88	88	88
Crude ash, max (%)	8	8	8	8
Crude protein, min (%)	19	18	16	18
Metabolic energy, min (kcal/kg)	2900	2800	2700	2800
Calcium, min-max (%)	1-1.2	1-1.1	0.9-1	3.5-4
Available phosphorus min (%)	0.45	0.42	0.40	0.40
Lysine, min (%)	1.15	0.98	0.72	0.75
Methionine, min (%)	0.55	0.47	0.35	0.47
Methionine+cystein min (%)	0.85	0.76	0.58	0.78
Tryptophan, min (%)	0.20	0.19	0.17	0.20
NaCl, min-max (%)	0.35-0.50	0.35-0.50	0.35-0.50	0.35-0.50
Crude cellulose, max (%)	4.5	5	6	6
Linoleic acid, min (%)	1.5	1.25	1.0	1.7
A vitamin (IU/kg)	13 000	13 000	10 000	12 000
D_3 vitamin (IU/kg)	3 000	3 000	2 000	2 500
E vitamin (mg/kg)	20	20	20	20
K_3 vitamin (mg/kg)	2	2	2	2
B_2 vitamin (mg/kg)	5	5	5	5
B_{12} vitamin (mg/kg)	0.02	0.02	0.01	0.01
Niacin (mg/kg)	60	60	30	25
Manganese (mg/kg)	100	100	100	60
Zinc (mg/kg)	70	70	70	40
Iron (mg/kg)	40	40	40	40
Cupper (mg/kg)	7	7	7	7
Selenium (mg/kg)	0.2	0.2	0.2	0.2
Cobalt (mg/kg)	0.5	0.5	0.5	0.5

The study was carried out in three environmentally controlled poultry houses. Each house had a 3-tier battery type cage system with a capacity of 120 hens. The temperature was set automatically to 20±2 ^{0}C in the control group, 12±2 ^{0}C in the low temperature group and 32±2 ^{0}C in the high temperature group. The relative humidity was set at 40% and ventilation was provided as 6.5 m^3 per hour air circulation per kg of live weight. Chicks were transferred

to the poultry house following 16 weeks of rearing in the rearing houses to which a 10 h of environmentally controlled illumination was provided daily. The chicks were randomly distributed to cage tiers. One pullet was placed to each cage (29x43x51 cm in dimension). Illumination period was kept at 10 h per day until the pullet reached 18 weeks of age; afterwards it was increased by an hour a week and fixed at 16 h. Compact fluorescent lamps for illumination were placed with a distance of 120 cm from the cage on the middle tier in the manner to provide 4watt/m^2 of light intensity. Feeds were given *ad libitum* to the animals. Hens were subjected to heat stress for a period of 3 weeks after reaching 27 weeks of age. In the last two days of heat stress, quality traits given below were determined on a total of 540 eggs, with 180 eggs from each group.

-*Egg weight***:** Weight of each egg was weighed by a scale with a sensitivity of 0.01g,
-*Shape Index:* By means of shape index measurement instrument developed by Rauch,
-*Egg Shell Breaking Strength***:** By means of shell breaking strength instrument (Newton/cm^2),
-*Yolk Colour***:** In Roche scale value determined by digital yolk colour measurement instrument,
-*Shell Thickness***:** In mm, by means of micrometre,
-*Albumen Height:* Determined by tripod micrometre,
-*Haugh Unit:* Calculated by the use of egg weight and albumen height,
Haugh Unit = 100 Log (H+7.57 – 1.7 $G^{0.37}$*)*
H: Albumen height (mm)
G: Egg weight (g)
***Statistical Analysis*:** The data obtained from the study were evaluated by using Minitab 16 Package Statistics Program. Analysis of variance was conducted with the intention of determining whether the differences between groups were essential, Tukey multiple comparison method was used to determine the differences between group means.

Results and Discussion

Egg quality traits under cold and heat stress conditions of the present study are presented in Table 2. No differences were found between the temperature groups for shape index, albumen height and Haugh unit of the egg quality traits in the study (P>0.05). However, shell breaking strength, egg weight, shell thickness and yolk colour differed between the temperature groups (P<0.05).

Table 2. Egg quality traits

Egg quality traits	Heat groups		
	Control (20±2 ^{0}C)	Low (12±2 ^{0}C)	High (32±2 ^{0}C)
Shape index	78.244±0.181	77.900±0.214	77.278±0.850
Haugh unit	79.326±0.616	80.852±0.590	80.999±0.735
Albumen height (mm)	6.343±0.081	6.508±0.079	6.446±0.094
Shell breaking strength (N)	43.372±0.596^a	44.856±0.497^a	41.584±0.534^b
Shell thickness (mm)	0.337±0.002^a	0.335±0.002^a	0.323±0.002^b
Egg weight (g)	59.990±0.330^a	59.874±0.307^a	58.226±0.311^b
Yolk colour	11.278±0.050^a	11.400±0.069^a	10.804±0.078^b

Within columns, means followed by different letters are significantly different at $P = 0.05$; n=180; ± expresses the standard error

Egg shape index, Haugh unit and albumen height of the eggs did not change during 3 weeks of heat or cold stress in the present study (Table 2). These findings are not consistent with the results of Ahvar *et al.* (1982), Deaton, (1983), Gill and Gangwar, (1984), who found that

albumen height and Haugh unit decreased when hens were kept under the heat stress conditions. The difference between this study and other studies can be explained by the length of stress period, which might not be long enough to observe the negative effects of temperature stress especially at high temperature (32 ^{0}C).

Shell breaking strength, shell thickness, egg weight and yolk colour did not differ in hens under cold stress but substantially decreased under heat stress. Lack of difference between the hens in the control group and in the cold stress can be explained by the ability of hens to balance their body temperature through obtaining additional feed during cold environmental conditions. This probably did not cause a serious problem for the metabolic and productive activities of birds (Czaricik and Fairchild, 2008). However a limitation in the provision of feed to the hens under cold environments can likely result in effects, which are similar to those observed under heat stress conditions. The findings of hens under heat stress are consistent with those of Ahvar*et al.* (1982), Daniel and Bolnave, (1979), Deaton, (1983), Gill and Ganwar, (1984), Patterson, (2004) and Rozenboim *et al*. (2007), but contradict with the findings of Dai *et al.* (2009), which indicated that heat stress did not influence egg weight, shell thickness, shell breaking strength and yolk colour.

Conclusion

Heat stress decreased egg quality traits in ATAK-S layers, which would have a negative effect on both producers and consumers. In order to prevent such losses, the construction and management of poultry houses for ATAK-S layers, especially in regions with warm climate should carefully be planned and anti-stress measures should be taken.

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Review paper
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A STUDY ON THE PRESENT DISEASE SITUATION OF THE CULTURED RAINBOW TROUT (ONCORHYNCHUS MYKISS, W.) IN TURKEY

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Abstract

The aim of this study is to inform about the present disease sitiuation of the cultured rainbow trout (*Oncorhynchus mykiss*, W.) in Turkey. Aquaculture around the world has been increased during the last decades, leading to spreading of infectious diseases and economical losses due to fish mortalities and costs of treatments. Rainbow trout was first introduced Turkey in 1970s and then the culture of species has gained in 1980s. Nowadays, it is grown at the farms which are located in the different regions of Turkey. However, disease is one of factors that limits to fish production because of causing problems including morbities and mortalities. Disease outbreaks at the farm conditions can result from the introduction of pathogens or parasites, from physical and chemical alterations of the surrounding water, and from all these factors. Although infections due to Gram-negative bacterial species are commonly observed among the cultured fish species, reports on Gram-positive coccal infections have been increased during the last decade. Among infectious diseases, some of them are known as old diseases such as IPN, RTFS and Yersiniosis. These were reported in the rainbow trout farming in Turkey. Nevertheless, the rainbow trout culture has been increased, new pathogens and diseases are observed by producers and researchers.

Keywords: *Rainbow trout, Oncorhynchus mykiss, diseases, Turkey*

Introduction

Rainbow trout (*Oncorhynchus mykiss*, W.) is one of the economically important fish species. Farming of the species was first started in 1879 in the United States and then, it was introduced to another countries for food, restocking and angling purposes (FAO, 2012). In Turkey, the farming attempts of the rainbow trout began in 1970s; however, the principal developments on its culture were gained in 1980s. Because of the marked demand of it, the annual production of the species has gradually been increased from 38.500 t in 1999 to 111.335 t in 2012 (TUIK, 2012). One of the primary goals in aquaculture systems is to maintain fish in healthy condition. This helps that fish grow rapidly and they show high survival from egg to marketable size (Gatlin, 2002). Fishes are aquatic and poikilothermic animals. In oceans and seas, the conditions of water column are very uniform but ponds, hatchery facilities and aquaria in which fishes are grown have more variable conditions (Wedemeyer *et al*., 1976). If these conditions have advers effects in maintaining health of fish, the fish are under the stress and a result of stress, they become sick. This paper informs the present disease sitiuation, especially major infectious bacterial, viral and parasitic diseases of the cultured rainbow trout (*Oncorhynchus mykiss*, W.) in Turkey.

Bacterial Infections

This group includes diseases which are caused by Gram-negative bacterial pathogens including *Flavobacterium psychrophilum*, *Yersinia ruckeri*, non-motile *Aeromonas* species and Gram-positive bacterial pathogen, *Lactococcus garvieae*. Because of their economical important, these diseases have been studied extensively and informed by different authors in Turkey. *Flavobacterium psychrophilum* was commonly reported from the disease outbreaks in Turkey and it is etiological agent of cold water disease (CWD) and rainbow trout fry

syndrome (RTFS). This pathogen from the affected rainbow trout samples from the different rainbow trout farms which were located in Mediterranean, Black Sea and Aegean regions of Turkey was first reported by Balta in 1997 (Balta, 1997). Then, the outbreaks from rainbow trout rearing facilities at Sapanca, Marmara region in 1997 was informed by Korun (Korun, 1999) and later, the outbreaks were reported by different researchers in the country (Durmaz *et* al., 2012; İspir *et* al., 2004; Diler *et* al., 2003; Korun and Timur, 2001). There is not commercial vaccine against *F. psychrophilum* agent. The treatment is achieved by adding different antibiotics into feed, especially onset of the disease. However, use of antimicrobial agents for the control of the disease, leading to increasing antibiotic resistance to *F. psychrophilum* strains. For example, Boyacıoglu *et al*., (2015) reported the efficacy of oxytetracyline (OXY) dropped between the strains of *F. psychrophilum* and currently, florfenicol (FFC) is favorable drug against the bacterium and until this time, resistance to this drug has slightly informed from Turkey. Yersiniosis or Enteric redmouth disease (ERM) is an important infection of freshwater farmed fish. Etiological agent is *Yersinia ruckeri*. First outbreak was informed from cultured rainbow trout in the Hagerman Valley of Idaho, the United States in 1950s. Then, the disease had a wide geographical distribution and reported in most countries (Austin and Austin, 2012). In Turkey, ERM from commercially produced rainbow trout was first observed in 1991 and afterwards the disease outbreaks in many parts of the country have been reported (Şeker *et al*., 2012; Onuk *et al*., 2011; Altun *et al*., 2010). Although commercial vaccine is available, disease outbreaks are stil observed. Şeker *et al*., (2012) informed that *Y. ruckeri* infection posed a significant threat to the farms in the sample area included Elazığ and Malatya provinces in Turkey and it was necessary precautions. Motile *Aeromonas* septicemia (MAS) is a fatal disease of fish and the infection is caused by different motile *Aeromonas* species (Das *et al*., 2013). *A. hydrophila* and *A. veroni* biotype *sobria* are predominantly responsible for outbreaks in fish. Motile *Aeromonas* septicemia from rainbow trout at a fish production and research station in Çifteler-Sakaryabaşı, Marmara region was first reported by Baran *et al*. (1980) in Turkey. The etiological agent is *A. hydrophila.* Subsequent informations on disease outbreaks due to this bacterium were given by other researchers (Aksoy, 2009; Sağlam *et al*., 2006). *A. sobria* was reported from rainbow trout farms located in the Central and East Anatolia and Mediterranean regions of Turkey (Özer *et al*., 2009; Özkök, 2005). It is understood that *A. hydrophila* and *A. sobria* are among the predominant motile *Aeromonas* species from rainbow trout affected by MAS. Lactococcosis is a serious septisemic problem of freshwater fish species including rainbow trout (Fereidouni *et al*., 2013). *Lactococcus garvieae* is etiological agent and the bacterium was first isolated from rainbow trout in Turkey in 2002 and then, it was reported from rainbow trout which had been affected by natural outbreaks in Turkey (Özer *et al*., 2008; Kav and Erganiş, 2007; Altun *et al*., 2004; Diler *et al*., 2002).

Viral Infections

Viral infections are important because of causing economic losses in fish farms around worldwide. Major salmonid viral infections include Infectious Pancreatic Necrosis (IPN), Infectious Hematopoietic Necrosis (IHN), Viral Haemorrhagic Septicaemia (VHS), Infectious Salmon Anemia (ISA) and Epizootic Hematopoietic Necrosis (EHN) (Collet, 2014). IPN virus (IPNV) infection as a case report was first reported in Turkey in 2002 but epidemiological screening and the following studies after outbreaks of the disease started in Turkey in 2004 (Kalayci *et al*., 2012). To date, the virus has been detected in mature and inmature rainbow trout samples from commercial farms located at the regions of the middle and east Black Sea, east and southeast Anatolia, Mediterranean and Anatolia of Turkey from December, 2006 to March, 2011 (Gürçay *et al*., 2013; Albayrak and Ozan, 2010). According to these authors' studies, IPNV occurs in the ova and milk of the infected fish and also rainbow trout farms so fish transports from one farm to another should be taken strictly

control measurements otherwise the infection will introduce to rainbow trout farms which have IPNV free rainbow trout. VHS was first described in Germany in 1930s but identification of the virus was carried out in 1960s (Faisal *et al*., 2012). VHS virus (VHSV) generally results in an haemorrhagic syndrome with septicaemia with severe bleeding of internal organs (Gadd, 2013). VHSV was first isolated from the diseased rainbow trout fry from a farm located at Bolu province in Marmara region, Turkey in 2006 (Kalayci *et al*., 2012). After one year this outbreak, VHSV was reported from another farm located at the same province. According to the European Union Directive (No: 91/67), the strict control and quarantina measurements were applied and then, the virus was not isolated the fish from these farms (Kalayci *et al*., 2012). IHN is one of the most serious diseases of the salmonid fish. Occurence of IHNV in rainbow trout was investigated in Turkey from 2006 to 2007 but the virus was not isolated (Albayrak and Özan, 2010).

3.Parasitic Diseases

Rainbow trout plays a role as host for more than 23 protozoan and at least 169 metazoan parasites in the worldwide (Buchmann and Bresciani, 1997). From protozoan parasites, White spot disease (Ich) is important for salmonid fish. The disease was reported from rainbow trout which were cultured in the different geographical regions such as Black Sea, Mediterranean and Aegean of Turkey (Özer *et* al., 2010; Kayış *et al.,* 2009; Öğüt *et al*., 2005; Tokşen, 2000). Öğüt *et al*. (2005) reported that *I. multifiliis* was endemic in the eastern Black Sea region and adverse enviromental factors including reuse of the water which was not treated by disinfection methods, improper water circulation, insufficient water supply and tank cleaning could be responsible for this endemic situation.

Conclusion

The above mentioned diseases are widely observed and informed infections except some of them. These are known since the trout culture has been started in Turkey. However, new diseases were reported in Turkey although they have not been known whether they are endemic or not.

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ANALYSIS OF EFFECTIVE FACTORS ON THE DEVELOPMENT OF TURKISH POULTRY SECTOR

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Abstract

In Turkey, supply-demand fluctuations and price instability in alternative products, poultry sector has done in the foreground and sector, especially that it has rapidly developed in last years on broiler meat and egg. Turkish poultry sector is accepted industry together with these developments, modern facilities, production methods, thanks to international standards and international markets in close proximity, such as benefits, has advantages and can compete with the whole world. However, there are some problems such as the height of the production costs of the industry, dependence on external inputs such as raw materials in feed and breeders. Fortunately, Turkey is a country that can adapt very quickly to emergencies. Turkish poultry sector is also facing difficult conditions and trying to adapt to new and often succeed. The policies being followed for the elimination of dependence on foreign, measures are taken to reduce costs and the growth of the sector is supported in a stable manner to increase production and consumption. A sector of the problems encountered, so a closer cooperation with all partners in the industry to give serious economic losses to the country and more work. Thus, the Turkish poultry sector healthier, more power, showing rapid increase of the current employment and a planned development, the reduction of unemployment and finding a solution to the immigration problem is expected to become much more useful sector. Thus, the more healthy the Turkish poultry sector, developing fast and planned faster in further increase of current employment power, reduction in unemployment and finding a solution to the immigration problem is expected to become much more useful sectors.

Keywords: *Turkish poultry sector, broiler, egg, productivity.*

Introduction

Poultry industry in the world is contributing an amount increasing the supply of affordable animal protein. Poultry products, which occupies a strategic position in human nutrition, also has a special emphasis on red meat off the unstable nature of the production deficit. Poultry meat production in more than 50 countries around the world because of these features have economic importance (Roenigk, 1999).

"Poultry meat" are classified in five groups as chicken meat, duck meat, geese and guinea fowl meat, turkey meat, pigeon and other birds by Food and Agriculture Organisation of the United Nations (FAO). Due to almost all of the broiler and turkey meat is Turkey’s poultry production and the lack of an industrial production of meat that is considered important outside of these two groups, the review is given to the broiler and turkey meat.

Nowadays in Turkey, 91% of the production of poultry meat, hatching eggs and chicks, and 75% of the production of table eggs are carried out by a small number (respectively 29 and 45) of large capacity integrated firms (Koca, 2015). The majority of these facilities working with contract producing systems are newer and more advanced technology is used compared to developed country. Therefore, the quality of poultry products generated is very high and the quality of products produced on a very large number of developed countries. Determining as one of several sub-sectors of the Turkish poultry sector could compete with the EU in the

food sector and being one of the most well-organized food sub-sector, reveals the importance for the Turkey. In this study, actual developments, problems and solution alternatives on Turkish poultry sector are evaluated.

Developments in Turkish Poultry

Broiler meat production in Turkey generally increased regularly, while turkey meat production began to decline after 2005 began to rise again in recent years. Broiler meat production in Turkey is developing rapidly settled 8th place in the world as of 2013 (Anonymous, 2014a). 3300000 tons of broiler meat production and 95.000 tons of turkey meat are expected to reach in 2023 (Table 1).

Poultry meat consumption in Turkey, depending on developments in the sector level and red meat prices, especially in recent years has increased considerably. The increase in poultry meat consumption is largely due to the increase in consumption of broiler meat (Table 1).

Table 1**.** Development of Turkish Poultry Sector (Anonymous, 2015).

Years	2005	2010	2014	2023 (target)
Production, ton				
Broiler meat	978400	1423000	1894668	3300000
Turkey meat	53530	33000	48663	95000
Consumption, kg/person/year				
Broiler meat	13.6	17.9	21.0	27.0
Poultry meat	15.1	19.0	22.3	28.8
Poultry meat exports				
Ton/year	44974	151339	430544	1086800
Million USD/year	34	230	700	1960

Turkey's poultry meat exports almost all chicken meat exports. According to the data of 2013, Turkey ranks 4th in the world chicken meat trade with 4.1% market share (Anonymous, 2014a) and aims to settle for third place behind the US and Brazil. 2014 poultry meat exports amounted to 431 thousand tons and 700 million US dollars. About 82% of poultry meat exports are 8 countries. Iraq (63.97%), the Turkish Republics (11.52%) and Russia (5.79%) to come is one of the countries with the highest export (Koca, 2015). Between the years 2005-2014 about the product from 5% to 20% share of exports directed to the rising generation, is expected to exceed 30% in 2023 (Table 1).

Turkey's imports of poultry meat, is quite low when compared to exports. Turkey in the last 15 years 1996, 2000 and has been performing ever chicken meat imports in 2004, chicken meat were imported at very low values in other years.

Table 2. Development of Turkish Egg Production (Anonymous, 2014b).

Years	2006	2010	2014
Production, million			
Poultry Egg	8401	12737	17607
Consumption, total/person/year			
Poultry Egg	114	174	226
Egg Exports, million			
Quantity	195	2.143	4.649
Value, million USD	18.927	156.619	404.094

Egg production in Turkey is rapidly developing. Our country ranks 13th in the world in terms of egg production. Due to increased production and consumption, rapidly developing egg exports reached 4.649 million eggs and 347 million USD levels in 2014 (Table 2).
Besides meat and eggs, as well as the main chicken product, slaughter by products is used as feed ingredients in feed mills by processed in rendering plants such as poultry offal and feathers. Recently, chicken feet are exported to the Far East countries constitute a growing economic potential. Obtained manure, slaughterhouse and hatchery by-products during the activities in the sector can also be used in other areas of agriculture

Problems and Solutions in Turkish Poultry

In the world, 2008-2009 followed by a partial recovery of the global financial crisis, although seen, troubles in the euro zone is still continuing. However, the growth of the Turkish poultry sector in 2014 was 3.5% (9.2% increase in broiler meat production (Koca, 2015)) and the success of the sector's production and export growth can not to be disputed. In Turkish livestock sector, poultry sector is the most advanced and competitive sector. This success in the sector, carried out by means of a high amount of production and export and which is making an enormous effort to do this and are trying to overcome a lot of adversity. Although the rapid development in the Turkish poultry sector is also experiencing major problems. Problems in the Turkish poultry sector can be divided into four main sections as production, consumption, sales-marketing and legislative issues.

Production Problems and Alternative Solutions

A set of problems related to raw materials, house and breeding are considered as problems related to production. In particular, high raw material costs and dependence on external inputs, it is observed as the main difficulties preventing the development of the sector. Importing of corn, soy and fish meal which are main raw materials of poultry feed has led to increased dependence on the outside of the industry. In Turkey, especially inadequate in soybean production (need 2 million tons, production 200 thousand tons), while maize production is very expensive (US 1.5 times) due to the fact, come to import the agenda and continues related problems (Koca, 2015). Moreover, feed raw materials such as corn and soybeans, even if taken within the scope of inward processing regime (Inward Processing Authorization Certification/DIIB), is reported to be a major disadvantage of high shipping cost (Akbay et al., 2000 and Turkoglu et. al, 2010). In order to eliminate problems that might prevent the development of industry, primarily reducing feed costs make up a large portion of production cost is required. To do this, increase the amount of domestically produced feed stuffs, especially with the development of policy support in soy and corn may be helpful (Dastan, 2010). Base price application is another factor that increases the costs and the base price an end to the practice, with world prices + the bonus system started to be well worthwhile.
An insufficient number of poultry house is a major obstacle in front of the healthy production. When not enough growing houses, arrest the targeted production is very difficult. In 2014, the Instrument for Pre-Accession Assistance Rural Development Program (IPARD) grants and disbursed through the Ziraat Bankasi reduced interest in the investment and business loans, house investments have taken place and has set an important contribution to the state sector. Such supports the continuation of improved and will provide a reduction in the problems of production-induced house.
Activities related to breeding, is notable as one of the least important part of the poultry industry. One of the reasons is the high research and production cost of breeding. Lack of trained personnel and experts is another important reason. In the Turkey breeder production to eliminate the dependence on foreign, the establishment of public and private sector

organizations institutions to hold the hands of the breeder production and grant these organizations, supported by applications such as low-interest loans and tax exemption aimed to encourage investment (Akbay et al., 1995 and 2000). As a result of improvement works carried out under these objectives, ATABEY, ATAK and ATAK-S is domestic laying lines have been developed. In 2013, the number of breeding domestic laying 25000, while the number of chicks laying sold reached approximately 500000. Even if efficiency levels up to foreign lines, production conditions in some facilities are not adequate for foreign hybrids, have been shown to yield positive results. The work initiated for domestic broiler breeder production is expected to be taken in the shortest time (Anoymous, 2013). To be successful, this project is supported investments are encouraged cooperation between public and private sectors. In addition, a set of environmental conditions inside the organization to take part in genetic improvement in the public sector, are arranged according to today's technology, lack of staff and experts are trying to resolve.

Consumption Problems and Alternative Solutions

Although the consumption of poultry meat increased significantly in recent years in Turkey is lower than the consumption of the major producing countries is another problem that needs to exceed the industry. To increase the Turkey's domestic consumption and export, it is necessary to increase the supply of further processed poultry meat products with different-flavoured, spicy and ensure the supply of different poultry species meat products (Keskin and Demirbas, 2012).

Media-related problems and information pollution are among the most important issues affecting consumption. In the written and visual media, without any scientific basis for the information and explanations which cause pollution in the community, causing serious damage to the sector. Development of the poultry industry, creating facilities that will contribute to companies and providing of public sector-private cooperation, many studies are performed by different organizations. Turkish Poultry Meat Producer and Breeders Association (BESD-BIR), Turkish Egg Producers Association (YUM-BIR) and Turkish Feed Manufacturers Association (TURKIYEM-BIR) are the most important of this organizations.

Social media are monitored and significant improvements are reported in order to reduce media-related problems and information pollutions, improve communication and inform the public and press. Press releases are issued in consultation with subject matter experts and PR activities are carried out. Together with PR work, as opposed to unfair and inaccurate perceptions about the sector's existing knowledge and perception of pollution has been started work to translate. The perception surveys are performed to demonstrate the consumption habits in the industry. The research results are evaluated and policy are determined accordingly. The results of the studies are recorded significant progress in solving the problems related to consumption.

Sales & Marketing Problems and Alternative Solutions

Problems with exports are among important factors limiting the development of the sector. To achieve the export target, it is necessary to develop existing markets and reach new markets. As a result of a variety of political reasons not affect the poultry meat exports, Turkey has suffered in unable to use the potential and enter new markets. Brazil dominate some markets such as the Middle East and Russia makes it difficult for Turkey's entry into this market. Although it is ready for the legal procedures relating to exports, cheap raw materials, labour and energy costs cannot be engaged in the production and compete with Brazil. However, geographical proximity to the Russian market and that Muslim countries in the Middle East market, may be an advantage for Turkey. If it can be used effectively these advantages, Turkey are believed could receive a larger share of the Middle East and Russia market. For

example, Saudi Arabia, with over 800000 tons of chicken meat imports in 2014 are among the countries with the highest import. The region has become very important for Turkey from 2012 (Anonymous, 2015). Russia is an important market for Turkey and a very large part of Turkey's exports to Russia are mechanically deboned poultry meat (MDM). Russia, along with the US and EU to stop food imports, our exports to this country began our revival. To further increase exports to Russia, it is necessary to make some bilateral agreements and increase the quota (Turkoglu et al., 2014 and Koca, 2015).

Exports to China, Japan, and the Philippines is very important for Turkey. Preliminary studies have been completed in these countries, Japan is the breast meat importer such as the EU and China is chicken feet importers, it is important in terms of the increase in unit export prices. In particular, due to the Chinese export made with brokers and high transport costs, the economic loss in 2014 is estimated to be around 13.6 million USD (Koca, 2015). The development of trade with these countries, which will reduce losses and will encourage effortless reach new markets. EU is also a target market for more than 10 years, however, exports to this region with legal permits a kind of incomplete.

Export incentives are among the most important issues affecting the sales and marketing. Export support is available for $ 26 per ton broiler meat and $ 15 per 1000 table eggs. It is believed that in order to attain the target countries should be given special support exports and the sector for export subsidies should be increased (Turkoglu et. al., 2010 and 2014). At the beginning of 2011, was established to increase the export of Poultry Promotion Group (Turkish Poultry) of increasingly more successful activities are also expected to contribute to development.

Legislation Problems and Alternative Solutions

The problems concerned with legislation are very important issues for the sector. Use of mechanically deboned meat (MDM) in some further processed poultry meat products ban, use of animal by-products (rendering products) in poultry feed ban, the difficulties in the importation of GMO-containing feed stuffs, the small number of reference laboratory, health issues such as the presence of Salmonella in poultry products, the lack of R&D projects and related supports are the most important problems related legislation.

Turkey, which is still in force, the use MDM ban in some further processed poultry products like sausages, nor the EU, nor the USA nor in any other region of the world is not prohibited. Therefore, improving the regulations on the use of MDM shall be made as soon as possible for the holders of both country and industry with consumers. The using ban of rendering products will also lead to additional costs and foreign exchange losses. In this regard, it is believed that it was necessary to postpone the application. Also, evaluate and develop waste projects all over the world to make the food, some groups in our country do not want to become waste of food is also considered to be a significant problem. The risk of contamination depends on the GMO feedstuffs, analyse errors and problems due to the difficulty of importing new development sector puts too much trouble. Increasing the number of reference laboratory as soon as possible will reduce the waste of time and money in the sector. Manure subject, for not properly managed, consistently stands out as a problem in front of the Turkish poultry sector. To solve the problem as soon as possible is being done to continue negotiations with relevant ministries.

Conclusion

In the poultry sector, as in all sectors, target, to obtain more products with low cost, high quality and healthy, and markets these products at high prices by ultimately achieve the highest profit possible. Turkish poultry sector has advantages in terms of factors such as modern facilities, international standards and can compete with the whole World, appropriate production methods and the proximity to the international market. But the height of

production cost feed raw materials and foreign dependency in feed stuffs and other outputs is limiting case for the future of the sector. In addition, factors such as infectious disease, can negatively affect industry and can change the market preferences.

Monitoring of policies to eliminate dependence on foreign, of taking measures to reduce costs and to increase the domestic consumption sector are expected to support growth in a stable manner and the positive impact that sector.

Republic of Turkey Ministry of Food Agriculture and Livestock, the objective of the policies implemented in the poultry sector, to increase per capita poultry meat and egg consumption to the European countries level by consumer education and advertising has determined to bringing to work at full capacity established facilities and increasing to exports (Anonymous, 2013. In line with these objectives, studies are carried out in the private sector and government cooperation and with great sacrifice to perform suitable for animals, economic and controlled care and feeding. Thus, both the economic efficiency and more comfortable poultry production with high-quality meat production of poultry will be possible.

Turkey is a country that can adapt very quickly to emergencies. Turkish poultry sector is also facing difficult conditions and trying to adapt to new and often succeed. The problems encountered in the sector and thus causing serious economic losses to the country, it is thought that a closer cooperation with all partners in the sector and further work is required. The problems encountered in the sector, does not cause serious economic loses in our sector and our country, it is thought that more effort is needed.

Turkish poultry sector is healthy, with rapid and planned development, when the obstacle is reduced, a further increase of the current employment force, the reduction of unemployment and finding a solution to the immigration problem will become much more useful one sector.

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DOG FIGHTING IN EUROPE

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Abstract

Aim of this paper is to revise dog fighting in Europe. Dog fighting has been illegal in Canada since 1892. Nowadays in USA dog fighting is illegal in all 50 states. Dog fighting is also illegal in most South American countries. In Afghanistan although dog fighting is banned by the Taliban it is still common throughout Afghanistan as a winter weekend pastime. In India dog fighting is not common, but can be found in some rural areas, and is illegal as defined by the Indian law. In Japan dog fighting seemed as legal. Dog fighting was considered a way for the Samurai to retain their aggressive edge during peaceful times. In Pakistan dog fighting and other forms of animal fighting are highly popular in all parts of the country even though it has recently been banned. It is deeply rooted in the rural culture. In Russia dogfighting is prohibited in much of the West. These blood sports were officially eliminated in 1835 as Britain began to introduce animal welfare laws. In Africa the Republic of South Africa is a country where one of the most dog fighting events happens. Dog fighting and the possession of any fighting equipment designed for dog fighting is illegal in Australia. "Restricted Breed Dogs" cannot be imported into Australia.

Keywords: *Canis familiaris, Pit Bull, blood sport, illegal sports, animal fight.*

Introduction

Domestic livestock animals serve humanity by giving plenty of products including meat, milk, egg, honey, floss silk, wool, fur, skin, feather, intestine, work, amusement etc (Ertugrul et al., 1993). Moreover some animals have been and continue to be used for fighting by some mentally abnormal people. In various regions of the world there are popular animal fighting events including fighting of bull, camel, cock, cricket, dog, horse, partridge and ram (Köhne and Ewigleben, 2000; Kalof, & Taylor 2007). For centuries usually dog fighting has been principally enjoyed by men. Unfortunately, the popularity of this activity has increased (Anon, 2014a). There was little initial interference from the law, since it was possible to fight two dogs in any hollow or shed without attracting much attention, for dogs fight with relatively little noise. They can easily be removed after the fight, carried away in sacks if their condition was likely to draw suspicion (Drable, 2014). For example in USA dog attacks and bites are not so common (Hussain, 2005). Although more than 20 fatalities happened each year in USA because of dog biting, there was no report that a man did during dog fight, but the dog. Maybe the 20 fatalities because of dog biting seem as tragic but at least 8.000 humans were killed by other human each year which was 200 times more than dog biting deaths (Lockwood, 1995).

The purpose of this paper is to review dog fighting in European countries.

Dog Fighting in the World

Dog fighting has been illegal in North America and under police surveillance but in South America there are some dog fighting events in some countries (Anon, 2014b). Nowadays in USA dogfighting is illegal in all 50 states (Anon, 2014c, Villavicencio, 2007, Kalof and Taylor, 2007).

In Asia dog fighting is quite common. Although it is one of the poorest countries in the world, animal cruelty arguments don't carry much weight in Afghanistan (Villavicencio, 2007; Anon, 2014a; Motlagh, 2014). In India dog fighting is not common, but can be found in some rural areas. In Japan dog fighting seemed as legal (Villavicencio, 2007; Ensminger, 2010; Anon, 2014d,e,g). In Pakistan dog fighting and other forms of animal fighting are highly popular in all parts of the country. Generally two breeds are raised for the purpose, such as the Bully Kutta and Gull Dong (Longbottom, 2011; Nizami, 2012; Longbottom, 2011). In Russia dogfighting is prohibited in much of the West. Even though animal cruelty laws exist, dog fighting is widely organized in some part of the country (Anon, 2015a). The breeds of Caucasian Shepherd Dog, Georgian Shepherd, Central Asian Shepherd Dog, and Wolfhounds are used for dog fighting (Chivers, 2007; Villavicencio, 2007; Anon, 2014e).
In Africa the Republic of South Africa is a country where one of the most dog fighting events happens (Anon, 2014e, h, i).
Dog fighting in Australia is illegal but dog fighting is often associated with gambling activities and other illegal practices such as drug dealing and firearms (Anon, 2014e, h, j).

Dog Fighting in Europe

These blood sports were officially eliminated in 1835 as Britain began to introduce animal welfare laws. Since dogfights were cheaper to organize and far easier to conceal from the law than bull or bear baits, blood sport proponents turned to pitting their dogs against each other instead. Dog fighting was used as both a blood sport (often involving gambling) and a way to continue to test the quality of their stock. For decades afterward, dog fighting clandestinely took place in pockets of working-class Britain and America. In recent years the inner cities, especially in London have seen a steady rise in the number of convictions for dog fighting, primarily among teenage youths of Pakistani descent (Anon, 2014e). A research study was carried out to discuss financial aspects of illegal dog-fighting in the UK and to reflect upon and discuss the difficulties of researching illegal entrepreneurial activities such as dog-fighting which were operated for criminal profit. According to the study dog fighting activities were conducted by urban criminals often in a rural setting. Those crimes invariably occurred in a closed social environment to which the authorities and the academic researcher cannot legitimately gain access. In the case study the illegal activities can legitimately be regarded as being an entrepreneurial activity as they entail trading in a Kirznerian sense as well as financial implications associated with gambling (Smith, 2011).

Rules

Among animals the dog is well known that dogs are known as very loyal animals. Humans abuse this quality of the dog (Kalof and Iliopoulou 2011). A dog's bond to humans is analogous to child-parent and chimpanzee-human attachment behavior, because the observed behavioral phenomena and the classification are similar to those described in mother-infant interactions (Topal et al. 1998).
Dog fighting is described as “The act of baiting two dogs against one another for entertainment or gain” (Evans et al. 1998). It involves placing two dogs in a pit until one either quits or dies. Dog fighting is almost universally illegal. Generally, two dogs, often of a pit bull breed, are put into an area to fight and sometimes kill each other. Dog fights usually last until one dog is declared a winner, which occurs when one dog fails to attack under the rules of such events, one dog dies, or one dog jumps out of the pit (Iliopoulou 2014). Dog fighting can involve high stakes, and carries with it the same sociological dangers of other gambling, particularly illegal gambling, activities (Anon 2014b). In some organized fights, the fighting dogs are weighed to make sure they are approximately the same weight. Handlers

will often wash and examine the opponent's dog to remove any toxic substances that may have been placed on the fur in an attempt to deter or harm the opposing dog (Anon 2014d).
When the fight begins, the spectators place bets with one another on which dog will win. There are some strict rules for fights. The dogs continue fighting until one of them makes a "turn," which is defined as turning the head and shoulders away from the opponent. When the referee calls the turn, the handlers then handle their dogs when they are out of "holds," which means they are not biting each other. The dog who made the turn must "scratch" to his opponent. Scratching is defined as crossing the scratch line, which is drawn in the center of the pit, and attacking one's opponent within a specified amount of time usually 10 to 30 seconds. If a dog fails to scratch, his opponent is declared the winner. If the scratch is successfully completed, the fight continues. From this point on the dogs are handled only when they are out of holds, and the dogs are required to scratch in turn. If a dog fails to scratch in turn, he is declared the loser. The fight may end in one of four ways: first a dog's failure to scratch; secondly the owner's calling the fight (analogous to the manager of a boxer throwing the towel into the ring, a signal that the fighter has quit); thirdly the death of one or both dogs, or fourthly one of the dogs jumping the pit (Forsyth and Evans 1998, Anon 2014d, Gasimzadeh 2014). Fights can last either just a few minutes or several hours depending on condition of dogs. Both animals may suffer injuries, including puncture wounds, lacerations, blood loss, crushing injuries and broken bones. Although fights are not usually to the death, many dogs succumb to their injuries later (Anon 2014d).
Dog fighting events also help to spread some diseases such as *Canine babesiosis* and *Babesia gibsoni*. *C. babesiosis* and *B. gibsoni* spread during dog fighting because of non-vectored transmission by blood exchange during fighting and biting (Irwin 2009).

The Dogs

Many dog breeds have been bred specifically for the strength, attitude, and physical features that would make them better fighting dogs. Some traits are common among most of fighting breeds that it’s outward appearance, a large, stocky, heavy breed with a powerful build and strongly developed head, and threatening voice (Massey 2012). Drable (2014) reported from Vesey-Fitzgerald that early fighting dogs were of all sorts of shapes, sizes and colors since their breeding was very promiscuous. In 1860s they were two groups, from one of which the English Bull Terrier was developed and from the other the Staffordshire Bull Terrier. The both breeds were initially very game, since nobody would keep a bull terrier which was not, but men soon bred the English variety for show, and looks were "improved" at the expense of courage. The Staffordshire bull terrier continued to be bred for the pit and, though not very standardized even yet there is no living breed so game (Drable 2014).
In USA about 30 percent of all dogs in animal shelters are pit bulls, the breed used for dogfighting. In some areas this figure can climb to 60 percent (Villavicencio 2007). For professional and hobbyist dogfighters, the sale of pups from parents who have won several fights is a major part of their activity. Underground dog fighting publications and websites are commonly used to advertise pups or the availability of breeding stock. Many "street" fighters think they can also make money by breeding and selling dogs, but a great number of these animals are killed or abandoned if they fail to perform (Anon 2014d).
Generally all kinds of dogfighters crop the ears and dock the tails of fighting dogs. There are two main reasons. First it limits the areas of the body that another dog can grab onto in a fight. Secondly it makes it more difficult for other dogs to read the animal's mood and intentions through the normal body language cues dogs use in aggressive encounters. Fighters usually perform this cropping or docking operation themselves using crude and inhumane techniques (Anon 2014d).

Early dogs of the bull terrier type were bred for the working characteristic known as gameness, with the pitting of dogs against bear or bull testing this attribute along with the strength and skill of the dog. These early "proto-staffords" provided the ancestral foundation stock for the Staffordshire Bull Terrier, the Bull Terrier, the American Pit Bull Terrier and American Staffordshire Terrier. This common ancestor was known as the "Bull and Terrier" (Anon 2014j). Generally the breeds of Staffordshire Bull Terriers, American Pit Bull Terriers, American Staffordshire Terriers, and Bull Terriers are called as pit bulls (Massey 2012). In other countries breeds of Bully Kutta, Caucasian Ovtcharka, Central Asian Ovtcharka, Dogo Argentino, Fila Brasileiro, the Presa Canario, the Tosa Inu, and Turkish Kangal (Karabash) Shepherd are used for fighting (Gibson 2005, Ensminger 2010, Anon 2014d, Gasimzadeh 2014, Anon 2015b). Also some breeds are accepted as extinct such as Blue Paul Terrier, Bull and Terrier, Cordoba Fighting Dog, Dogo Cubano, English White Terrier, Molossus, Old English Bulldog dogs (Anon 2014d).

Table 1. Some breeds which are used for dogfighting (Anon 2015b).

Breed	Origin		Breed	Origin
Aryan Molossus	Afghanistan		Gull Terrier	Pakistan
Kuchi	Afghanistan		Dogue de Bordeaux	Portugal
Dogo Argentino	Argentina		Caucasian Ovtcharka	Russia
Armenian Gampr	Armenia		Central Asian Ovtcharka	Russia
Shar Pei	China		Alano Español	Spain
Tibetan Mastiff	China		Perro de Presa Canario	Spain
Georgian Shepherd	Georgia		Perro de Presa Mallorquin	Spain
Guatemalan Dogo	Guatemala		Kangal Dog	Turkey
Glen of Imaal Terrier	Ireland		Bull Terrier	UK
Lottatore Brindisino	Italy		English Mastiff	UK
Neapolitan Mastiff	Italy		Staffordshire Bull Terrier	UK
Akita Inu	Japan		American Pit Bull Terrier	USA
Tosa	Japan		American Staffordshire Terrier	USA
Korean Jindo	Korea		American Bulldog	USA
New Guinea Singing	Papua New Guinea		Boston Terrier	USA
Bully Kutta	Pakistan			

It is often asked whether the Pit Bull is unsuitable as a family pet or not? In the early 1900s pit bulls were considered the epitome of the all American dog. For example Stubby, the first war hero dog and Pete the Pup, from “The Little Rascals” were very good examples of this breed (Hussain 2005). Although bred for fighting other dogs the American Pit Bull terrier has long been a popular family pet, noted for his strength, intelligence and devotion. It is clearly understandable that any dog can behave aggressively, depending on the context, his genetic background, and his upbringing and environment. If a dog is treated well, properly trained and thoroughly socialized during puppyhood and matched with the right kind of owner and household, it is likely to develop into a well-behaved companion and cherished member of the family. However, some Pit Bulls and Pit Bull crossbreds may be more inclined to develop aggression toward other dogs (Anon 2014d).

American Pit Bull Terrier is a wonderful dog breed, but it comes with risks. Being large-jawed, it is more capable than many breeds of doing damage with its bites. As with any dog, pit bulls can be trained, and often are as calm as dogs can be, but people assume they are vicious because they have become popular with gangs and other unpopular members of American society (Ensminger 2012).

Even though German Shepherd Dogs (GSD) do not seem a fighting dog breed, they are actually a biteable dog breed. Lockwood reported from a study realized by Berzon that GSDs made up 45% of the dogs listed in Baltimore bite reports. Lockwood also reported from a study realized by Moore about percentage of the registered population of various breeds that were involved in bites. The highest rankings in that research were pit bulls (12.3%), chow chows (11.4%), GSD (6.5%), Dobermans (4.3%) and Rotweilers (4.1%) (Lockwood 1995). A study claimed that the annual incidence of dog bites was 0.5 percent 1000 children between 0 and 16 years of age. The relative risk for a dog attack by a German shepherd or a Doberman was 5 times higher than that of a Labrador Retriever or cross-breed (Schalamon et al. 2005).

Train

According to fighting dog trainer fighting dogs must be kept isolated from other dogs, so they spend most of their lives on short, heavy chains, often just out of reach of other dogs. They are generally unsocialized to any other dogs and to most people. However, many professional fighters invest much time and money in conditioning their animals. They are frequently given quality nutrition and basic veterinary care. The dogs are exercised under controlled conditions. The maintaining condition of fighting dogs may also make use of a variety of legal and illegal drugs, including anabolic steroids to develop muscle mass and encourage aggressiveness. Narcotic drugs may also be used to increase the dogs' aggression, increase reactivity and mask pain or fear during a fight. Young animals are often trained or tested by allowing them to fight with other dogs in well-controlled rolls. Any dog may be discarded or killed, if the dog shows little inclination to fight. Some fighters will use stolen pets as bait dogs or sparring partners. There are many other common techniques used in the training and testing of dogs, but these methods vary widely among different fighters and may range from systematic to haphazard. In USA the street fighters usually make little investment in conditioning or training their animals. Instead, they rely on cruel methods to encourage their dogs to fight, including starvation, physical abuse, isolation and the use of stimulants or other drugs that excite the dogs (Anon 2014d).

In USA fighting dogs are commonly conditioned from a very early age to develop what dog-fighters refer to as “gameness.” Dog-fighter applies training by depending on his level and experience on dogs. In USA the methods are commonly used to train the dogs:

Treadmill: The fighting dogs are run on the treadmills to develop cardiovascular fitness and endurance.

Catmill or Jenny: This device looks like a carnival horse walker with several beams jetting out from a central rotating pole. The dogs are chained to one beam and another small animal like a cat, small dog, or rabbit, is harnessed to or hung from another beam. The dogs run in circles by chasing the lure. Once the exercise sessions are over, the dogs are usually rewarded with the bait they had been pursuing.

Springpole or Jumppole: It is a tool that a large pole with a spring hanging down to which a rope, tire, or animal hide is affixed that the dogs jump to and dangle from for extended periods of time. This strengthens the jaw muscles and back legs.

Flirtpole: A handheld pole with a lure attached. The dogs chase the lure along the ground.

Chains: Dogs have very heavy chains wrapped around their necks, generally in lieu of collars; they build neck and upper body strength by constantly bearing the immense weight of the chains.

Weights: Weights are often affixed to chains and dangled from the dogs’ necks. This builds neck and upper body strength. Generally, dogs are permanently chained this way. However, sometimes the trainers run them with their weights attached.

Bait: Animals are tied up while the dogs tear them apart or sometimes they are confined in an area to be chased and mauled by the dogs.

Drugs, Vitamins, and Supplements: Dogs are given iron/liver extract; vitamin B-12; Provim; Magnum supplement; hormones of testosterone, Propionate, Repotest, Probolic Oil; weight-gain supplements; creatine monohydrate; speed; steroids of Winstrol V, Dinabol, Equipose; and cocaine. The dogs are trained against one another and against older, more experienced dogs. In the early stages of training, the dogs are incited to lunge at each other without touching and engage in quick, controlled fights called rolls or bumps. Once the dogs appear match ready, they are pitted against stronger dogs to test their gameness or tenacity in the face of exhaustion and impending defeat. If the dogs pass the test, they are deemed ready to fight (Gibson 2005).

Types of Fighters

There are three types of dog fighters including street fighters, hobbyists, and professionals. Hobbyists and professionals often decry the techniques street fighters use to train their dogs. Such techniques include starving, drugging, and physically abusing the dog. Street fighters are frequently associated with gang activities. They fight dogs over insults, turf invasions, or simple taunts like "My dog can kill your dog”. These type of fights are often spontaneous, unorganized, conducted for money, drugs, or bragging rights. Urban street fighters generally have several dogs chained in back-yards, often behind privacy fences, or in basements or garages. The dogs are often found by police and animal control officers either dead or dying after a street fight. Due to the spontaneity of a street fight, they are very difficult to respond to unless reported immediately. The second types of fighters are hobbyists. They fight dogs for supplemental income and entertainment purposes. They typically have one or more dogs participating in several organized fights and operate primarily within a specific geographic network. Hobbyists are also informed with one another and tend to return to predetermined fight venues repeatedly. The last types are professionals. Professionals breed generations of skilled "game dogs" and take a great pride in their dogs' lineage. Those fighters make a tremendous amount of money charging stud fees to breed their champions. They also earn the fees and winnings they collect for fighting them. Professionals also tend to own a large number of dogs which are sometimes 50 dogs or more. In USA professional fighters also use trade journals to discuss recent fights and to advertise the sale of training equipment and puppies such as Your Friend and Mine, Game Dog Times, The American Warrior, and The Pit Bull Chronicle. Some fighters operate on a national or even international level within highly secret networks (Gibson 2005, Ensminger 2010, Boucher 2011, Anon 2014b,d).

Place

Dog fights are organized in places of variety of locations and at any time. In rural areas they may be impromptu events in a barn, outdoor pit, back alley, or carefully planned and staged enterprises in a location specially designed and maintained for the purpose. On the other hand in urban areas, fights may happen in garages, basements, warehouses, abandoned buildings, back alleys, neighborhood playgrounds, or even in the streets (Kalof and Talor 2007, Anon 2014a,d). In USA two dogs are put into a square pit, which measures from 3.5 to 6 meters on each side. The pit is surrounded by walls between 0.6 and 1.2 meters high and usually made of wood, plywood, hay bales, or chain link. There are two handlers and one referee in the pit with the dogs. During dogfight spectators surround the pit, the sides of which are wooden and three to four feet high. The dogfight starts when the referee tells the handlers to pit their dogs, at which time the dogs are released and attack (Forsyth and Evans 1998, Anon 2014d).

Conclusion

It can be said that it is too difficult to understand that some people provide dog fighting events. According to those abnormal people dog fights are necessary and should be carried

out (Gasimzadeh 2014). This kind of people is rare in community but they are always present in the community and always will be. The most important thing to prevent dog fighting is inform related police or gendarme forces. Also penalties should be more increased and dog fighters should be published in TVs.

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PREDICTION OF POST-BROKEN TRAITS USING PRE-BROKEN TRAITS AS REGRESSORS IN GUİNEA FOWL EGGS

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Abstract

The research was conducted on eggs of Guinea fowl maintained in Department of Animal Science, Faculty of Agriculture, Akdeniz University, Turkey, in 2012. One hundred eggs selected randomly. Egg weigth (g), egg lenght (mm), egg width (mm), shell weight (g), shell thickness (mm) and yolk weight (g) were measured. The egg weight was beter predictable from egg width and egg lenght. Following equation was developed to predict egg weight from egg width and egg lenght. Y=-65.187*egg lenght+2.021*egg width. Folowing equation was developed for predicting shell weight from egg weight. Y=-2.507+0.224*egg weight. Egg shape index was predictable with sufficient accuracy from egg lenght and egg width and, following equation was developed to predict it; Y=0.752-0.015*egg lenght+0.021*egg width. Eggshell thickness was beter predictable from shell weight. So, following equation was developed for predicting shell thickness. Y=0.232+0.048*egg weight. Yolk weight was predictable with sufficient accuracy from egg weight and following equation was developed to it; Y=3.898+0.241*egg weight. Weight of egg albumen could be predicted from the following equation, Y=-30.438+1.374*3gg width. Followig equation was developed for predicting yolk height from egg weight, Y=9.871+0.128*egg weight. Eggshell surface area was predictable with sufficient accuracy from egg weight and following equation was developed to predict it; Y=18.85+0.918*egg weight.

Keywords: *Guinea fowl, egg weight, egg lenght, egg width, shell thickness*

Introductıon

Guinea fowl (*Numida meleagris*) are native to Africa and are believed to have originated in Western, Central and Southern parts of Africa (Ikani and Dafwang, 2004; Fajemilehin 2010; Tebesi *et al.*, 2012, Elhashmi *et al*., 2012). Although Guinea fowl are thought to have originated in Africa, its production has increased rapidlythroughout the entire world. Guinea fowl can be successfully reared under semi-intensive conditions with less effort (Seabo et al., 2011). That is why it has been very important for Third World Countries. With the help of developments in organic farming rechnigues in recent years the Guinea fowl has been given importance as altenative poultry (Yildirim, 2012).

Their meat and eggs are very popular and carcass weights between 0,5 and 1,0 kg at 10 weeks of age making them suitable table birds for the small family. The demand for guinea fowl meat in the market has significantly increased due to flavour making it to be serves extensively in the larger hotels and higher priced restaurants (Feltwell, 1992). Guinea fowl represents a reliable and active contributor to the survival of the rural folk since it has economic, cultural, livelihood and social values in their lives (Tye and Gyawu, 2001). In Zimbabwe today, guinea fowl is now being viewed as a potential vehicle in the reduction of rural poverty, a strategy already operational in Ghana (Ghanadot 2009; Kusina 2012). These fowls have kept their characteristics until today such as being matured, reproduced and resistant to diseases under different ambient temperatures (Kusina, 2012). Other advantages

are their need for less water and pratical semi-intensive breeding in the farms or around villages.

The quality of table or hatching eggs depends on a number of traits which include egg weight, egg lenght, egg width, eggshell weight, eggshell thickness, eggshell ratio, egg shape index, egg surface area, unit surface shell weight, yolk weight, yolk diameter, yolk height, yolk index, yolk ratio, haugh unit, albumen weight and albumen height if other management conditions and health status of the birds are not the limiting factors (Khurshid *et al.,* 2003; Fajemilehin, 2008). Among the traits mentioned above, egg weight, lenght and width are the only parameters which could be determined before breaking the eggs, while information on rest parameters are usually determined after the eggs have been broken.

Regression analysis is indeed the single most important useful measure of association which can be used to explain the amount of change in one variable as a result of a unit change in the value of other variable since we have control over the first variable and can measure it essentially without error (Glover and Mitchell, 2001; Fajemilehin, 2008). Egg weight was esaily predictable from egg lenght and width as positive association among these traits existed (Farooq *et al.,* 2001). This type of information on egg weight along with other egg parameters will poen the domain for trying out various prediction equations in order to predict each of the parameters. Prediction of egg quality traits prior to marketing for table is the prerequisite for economic production of eggs.

The research was carried out to predicting egg weight, shell weight, shell thickness, shell surface area, egg shape index, height of egg yolk, yolk width, yolk weight, albumen length and albumen weight using various egg traits as independent variables.

Materıal and methods

This study was conducted on total 100 egg of Guinea fowl maintained in Department of Animal Science, Faculty of Agriculture, Akdeniz University Turkey, in 2012. During the research, 0.01 g sensitive electronic scale was used for weighing the eggs; a compass sensitive to 0.01 mm was used for measuring the length, width, yolk diameter, albumen length of the eggs; a table with a flat glass on it was used on which the eggs are broken; a 3-legged micrometer sensitive to 0.01mm was used for measuring the height of yolk and albumen and a micrometer sensitive to 0.01 was used for measuring the shell thickness. The collected eggs to be used in this research were numerated at first. Then, they were balanced in order to determine their weights. Later, the width and length of the egg were measured. After this process, the eggs were broken on table with a glass cover in order to measure yolk height, yolk diameter, albumen length and albumen height. The eggshells were washed under slightly flowing water so that albumen remains are removed. The washed eggshells were left to dry in the open air for 24 hours. Then, they were balanced together with the eggshell membrane. Finally, samples taken from sharp, blunt and equatorial parts were measured and the average shell thickness was obtained from the average values of these three parts (Tyler, 1961). The surface area, expressed as centimeters squared (cm^2), of each egg was estimated using the formula of Carter (1975), 3.9782 x W.7056, where W is egg weight in grams.

Shape index = egg width / egg length x 100

Following model was used for prediction of egg weight, using egg length and width as independent variables in different cases;

$Y_{ij} = a + b_iX_i + e_{ij}$

Where, Y_{ij} :egg weight (g), a: the intercept, b_i: the regression coefficients, X_i: egg width and length (mm) and

e_{ij}: The residual term.

A similar model was used for predicting other traits using egg weight, length and width as independent variables.

Results and dıscussıon

Predicting of egg weight from egg lenght and width

In this study, following equation was developed for predicting egg weight from egg length and width.

Y = -65.187 *Egg lenght + 2.021*Egg width

Where; Y = will be predicted egg weight

Predicting of eggshell weight from egg weight, egg length and width

Following equation can be effectively used for predicting eggshell weight from egg egg weight;

Y = -2.507+0.224*egg weight

Where; Y = will be predicted eggshell weight

Predicting of eggshell thickness from egg weight, egg length and width

Following equations were developed for predicting eggshell thickness from eggshell weight and egg weight.

Y = 0.232 + 0.048*eggshell weight

Y=0.196+0.09*egg weight

Where; Y = will be predicted eggshell thickness

Predicting of eggshell surface area from egg weight, egg length and width

Following equation was developed to predict eggshell surface area from egg weight.

Y = 18.85+0.918*egg weight

Where; Y = will be predicted eggshell surface area.

Predicting of egg shape index from egg weight, length and width

Following equation was developed to predict egg shape index from egg length and width.

Y = 0.752 - 0.015*egg lenght + 0.021*egg width

Y = will predicted egg shape index

Predicting height of yolk from egg weight, egg length and width

Following equation was developed to predict height of yolk from egg weight.

Y = 9871.908 + 0.128*egg weight

Y = will predicted height of yolk

Predicting width of yolk from egg weight, egg length and width

Following equation was developed to predict width of yolk from egg width.

Y = 17.0064+ 0.624*egg width

Y = will predicted width of yolk

Predicting weight of yolk from egg weight, egg length and width

Following equation was developed to predict weight of yolk from egg weight.

Y = 3.898+ 0.241*egg weight

Y = will predicted weight of yolk

Predicting weight of albumen from egg weight, egg length and width

Following equation was developed to predict weight of albumen from egg width.

Y = -30.438+ 1.374*egg width

Y = will predicted weight of albumen

Egg weight was predictable with sufficient accuracy from egg width and length as association of both traits with weight of the egg was significant (P<0.01). Regression coefficient of the fitted model was 79.30%. Khurshid *et al.* (2003) reported significant and positive association of egg weight with egg length and width of Japanese quails and regression coefficient was calculated as 31.86%. As reported by Alkan *et al.* (2008) egg lenght and width have significant effect on the egg weight of Japanese quails and the regression coefficient was calculated as 82.60%. Farooq *et al.* (2001) also reported positive association of egg weight with egg width and length of Fayumi eggs. In another research, Fajemilehin (2008) reported significant and positive relationship of egg weight with egg lenght and width of Quinea fowls and regression coefficients were calculated as 21.10% for egg lenght and 16.82% for egg width.

The eggshell weight, shell thickness and eggshell stiffness are important egg traits that can't be exactly predetermined until and unless eggs are broken. However, prediction equations can be developed to get information about these traits without breaking eggs. Some researchers had focused on the studies related to the increasing of the stiffness of the eggshell, because the quality of the eggshell has a vital importance for the laying force, embryo growth and the chick quality (Altan *et al.*, 1998). In this study, eggshell weight was found significantly (P<0.01) and positively associated with egg weight providing a good ground for predicting eggshell weight. In this study, adjusted R^2 of the fitted model was 42.70%. Khurshid *et al.* (2003) reported significant and positive association of eggshell weight with egg length and width of Japanese quails and regression coefficient was calculated as 7.01%. As reported by *Alkan et al*. (2008) egg lenght and width have significant effect on the egg shell weight of Japanese quails and the regression coefficient was calculated as 50.70%. In another research, Fajemilehin (2008) reported significant and positive relationship of egg weight with egg lenght and width of guinea fowls and regression coefficients were calculated as 7.40% for egg lenght and 8.20% for egg width and 8.40% for egg weight. Also, Farooq *et al.* (2001) who reported significant association of shell weight with egg width in Fayoumi eggs.

The eggshell thickness was predictable with sufficient accuracy (P<0.01) from egg eggshell weight and and egg weight. In this study, regression coefficients were 74.90% for eggshell weight and 20.50% for egg weight. But, Farooq *et al.* (2001) and Khurshid *et al.* (2003) also reported significant association of eggshell thickness with egg width. Also, Alkan *et al*. (2008) reported that there was a significant relationship of eggshell thickness with egg lenght of quails. As reported by *Fajemilehin* (2008) eggshell thickness was predicted with sufficient accuracy (P<0.01) from egg lenght, width and weight. Eggshell surface area was predictable with enough accuracy (P<0.01) from egg weight. Regression coefficient of the fitted model was 1.00%. Alkan *et al.(*2008) reported significant association of eggshell area with egg weight of Japanese quails and the regression coefficient was calculated as 99.40%.

Egg shape index was predictable with better accuracy from egg egg width and length. Regression coefficient of the fitted model was 97.90%. Alkan *et al.(*2008) reported significant association of eggs shape index with egg lenght and width of Japanese quails and the regression coefficient was calculated as 98.80%. Height of yolk was predictable with accuracy (P<0.01) from egg weight. Because, egg length and widtht have no significant effect on height of yolk. Regression coefficient of the fitted model was 8.50%. But, Alkan *et al.(*2008) reported significant association of yolk height with egg width of Japanese quails and the regression coefficient was calculated as 46.50%. Yolk width was predictable with enough accuracy (P<0.01) from egg width. Regression coefficient of the fitted model was 10.90%. But, Alkan *et al.(*2008) reported significant association of yolk width with egg weight, lenght and width of Japanese quails and the regression coefficient was calculated as 79.40%.

Weight of yolk was predictable with better accuracy (P<0.01) from egg weight and regression coefficient of the fitted model was 31.60%. Khurshid *et al*.(2003) reported

significant association of yolk weight with egg weight and width of Japanese quails and the regression coefficient was calculated as 60.54%. Fajemilehin (2008) also reported positive association of yolk weight with egg weight of Quinea fowl eggs and the regression coefficient was calculated as 60.00%. Weight of albumen was predictable with better accuracy ($P<0.05$) from egg width and the regression coefficient of the fitted model was 5.10%. Fajemilehin (2008) also reported positive association of albumen weight with egg weight and width of Quinea fowl eggs and, the regression coefficient was calculated as 72.75%.

Conclusıon

As a result, the egg weight was enough predictable when egg lenght and egg width were used as independent variables. However, egg lenght and egg width can be used individually for predicting of egg weight. The eggshell weight was predictable with sufficient accuracy from egg weight. Eggshell thickness was predictable with sufficient accuracy from egg weight. Also, egg shape surface area and yolk weight can be predictable enough from egg weight. Albumen weight was predictable from egg width ($P<0.05$). But, egg weight has no significant effect on the albumen weight. At the same time, yolk width was enough predictable when egg width was used as independent variable. Egg weight, lenght and width have no significant effect on albumen width.

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CHEMICAL AND NUTRITIONAL CHANGES IN SUNFLOWER SILAGE ASSOCIATED WITH MOLASSES, LACTIC ACID BACTERIA AND ENZYME SUPPLEMENTATION

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Abstract

This study was conducted to determine the effects of different silage additives on sunflower silage quality.The treatments were as follows: (1) control (C, no additive), (2) 5% molasses (M) (3) inoculation of lactic acid bacteria (LAB; 1.5 g/ton, a mixture of *Lactobacillus plantarum*and *Enterococcus faecium* applied at a rate of 6.00 log10 cfu LAB/g of fresh material),and 4: LAB+enzyme mixture 2 g/ton (LEN, *Lactobacillus plantarum* bacterium (6.00 log10 cfu/g) and cellulose (150000 CMCU/kg) and amylase (200000 SKB/kg) enzymes). Silage additives were mixed and stored in glass jars with 5 replicates for 90 d.There were no significant differences among the groups in terms of pH, ether extract (EE), acid detergent lignin (ADL) and hemicellulose (HEM). The water soluble carbohydrate (WSC) concentration was higher in the M group than those of other groups ($P<0.01$). Lactic and acetic acid concentration were low in the LEN group than others ($P<0.05$). In the LAB group, the dry matter (DM), crude protein (CP), crude ash (CA) and fleig point (FP) were lower than those of other groups ($P<0.01$). In the M group, the neutral detergent fiber (NDF) and acid detergent fiber (ADF) were lower and the total digestibility nutrient (TDN) and non-starch carbohydrate (NFC) were higher than those of other groups ($P<0.01$). The crude cellulose (CC) of M and LEN groups were lower than C and LAB groups. In conclusion, the inoculants (LAB and LEN) and molasses addition to sunflower crop before ensiling may positively affect different silage quality characteristics.

Key words: *sunflower silage, molasses, inoculant, silage quality*

Introduction

In ruminant nutrition, roughagesarecheap and good nutrient sources and essential for goodrumen function. Silage production has some advantages in the dairy farms. However, to provide superior quality silage, appropriate plant, appropriate harvest time, enough in easy water soluble carbohydrates (WSC), thengood ensiling and fermentation are necessary.

Corn silage production is very common in dairy or beef cattle and sheep and goat farms. However, sunflower silage is not common as corn silage in these farms. For corn production, it is necessary better soil properties, high climatic temperature and more irrigation compared to sunflower plant (Gonçalves et al., 1999). As an alternative, sunflower has a good ability for drought tolerance, resistance to cold and heat, adaptability to different climatic conditions high dry matter (DM) yields, and relative independence of latitude, altitude and photoperiod (Tomich, 1999). Sunflower silage has higher concentration of protein and fat compared to corn (Gregoire, 1999) and sorghum (Demirel et al., 2006) silages. However, when it late harvest, due to increase fiber content of sunflower silage, lowersilage quality and digestibility of material in ruminants (Demirelet. al., 2006; Ozduven et al., 2009).

Silage additives commonly have been using in many farms for improve silage fermentation and quality.There are many commercial bacterial inoculants, enzyme and organic acids and their combinations (Meeske and Basson 1998; Sucu and Filya, 2006). However, many

experiments were done in corn silage (Aksu et al., 2004; Baytok et al., 2005; Filya et al., 2006) but lesser experiments done with sunflower (Denek et al., 2004; Ozduven et al., 2009) and other silage materials (Ozduven et al., 2010).

The biological inoculants as silage additive are producing via*Lactobacillus* bacteria (LAB) and stabilized for silage additive and generally they increase lactic acid concentration and reduce pH, acetic acid, butyric acid and ammonia- nitrogen levels in silage (Aksu et.al., 2004). Last decade, there are many effort to produce new strainof homo and hetero fermentative bacterial inoculants by the commercial companies as an alternative to former inoculants and new combinations with enzymes. On the other hand, commercial biologic silage inoculants may be costly to farmers and some conditions may not reliable due to inactivated microbial organisms (Weinberg and Muck 1996). Molasses is a by-product of sugar production factories and it can be used to get water soluble carbohydrate source in silage (Nkosi et al., 2010).

The aim of this study was to evaluate the effect of molasses and biological silage additives on sunflower silage quality traits.

Material and methods

Sunflower plant material was obtained from Erciyes University Agricultural Research Field without any treatment and harvested at the late flower stage of maturity (31.10 ± 0.81% DM) at August17, 2013. The whole plants were chopped about 2 to 4 cm and ensiled in 1 kg capacity glass jars with 5 replications. The chopped fresh materials were filled tightly in order to avoid oxygen. The ensiled jars were stored at room temperature (20°C ± 3°C) for 90 days.The treatment groups were as follows: (1) control (C, no additive), (2) 5% molasses (M) (3) inoculation of lactic acid bacteria (LAB; 1.5 g/ton, a mixture of LAB consisting of *Lactobacillus plantarum*and *Enterococcus faecium* applied at a rate of 6.00 log10 cfu LAB/g of fresh material, Pioneer 1174, USA),and 4: LAB+enzyme mixture 2 g/ton(Inoculant and enzyme mixture (LEN, *Lactobacillus plantarum* bacterium (6.00 log10 cfu/g) and cellulose (150000 CMCU/kg) and amylase (200000 SKB/kg) enzymes, Silaid WS^{TM}, Global Nutritech Co., USA). Themolasses, LAB and LEN were dissolved in 20ml water and sprayed on the chopped sunflowerfresh materials.

Chemical analyses

At the end of the 90 days ensiling period the silages were sampled for chemical and other analyses. pH measurements were taken for 25 g of silage samples taken in a beaker by adding 100 ml of distilled water in a blender for 5 minutes then decomposed with silage pH was measured (Akyildiz, 1986). The DM content of the silages was determined by drying at 60°C for 72 h in an oven. Crude ash of samples was obtained after 4h at 600°C (AOAC, 1990). Crude protein (CP), crude fiber (CF) and ether extract (EE) were determined according to the AOAC (1990) method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analysed using the sodium sulphite addition method with residual ash (Van Soest et al., 1991). The difference between NDF and ADF values provides an estimate of hemicellulose. At the end of the ensiling period the silages were sampled as a liquid extraction for WSC and volatile fatty acid (VFA) analysis. The liquid extractions were prepared with 40 g silage to be put into a beaker and added 360 ml distilled water, than mix with a blander. This liquid was filtered through Whatman 54 filter paper and then centrifuged. Samples were stored at -20 °C until analyzed. WSCs were determined by the phenol-sulphuric acid method (Dubois et al., 1956). Lactic acid (LA) was determined by Lepper's methods (Akyildiz, 1986). VFA (Acetic, Propionic and Butyric acid) analysis were analysed in a gas chromatograph with an capillary column (30 m × 0.25 mm × 0.25 μm, Restek), in a Shimadzu GC-2010+ (Kyoto, Japan) gas chromatograph equipped with FID, over a temperature range of 45–230°C.

The total digestible nutrients (TDN) values were calculated according to the equation proposed by Chandler (1990), where %TDN = 105.2-0.68 x %NDF. The non-fiber carbohydrates (NFC) were calculated by the equation proposed by Weiss et al., (1992): %NFC = 100 - (%NDF + %CP + %EE + %CA). Total carbohydrates (TC) were determined according to Sniffen et al., (1992) by equation TC = 100 - (% CP +% EE +% CA). The metabolic energy (ME) was calculated by the equation proposed by Kirchgesser, (1997): 14.03-(0.01386 x % CF)-(0.1018 x % CA).

Statistical analysis

Data were analyzed using the general linear model procedure of the SPSS program. Differences between reported means were determined using the Duncan's multiple range tests with a 5% level of probability. The results of statistical analysis were shown as mean values and standard error of the means (SEM) in the tables.

Results and discussion

The effect of molasses, lactic acid bacteria inoculant and enzyme supplementation on the sunflower silage pH, dry matter, crude protein, crude cellulose, crude ash and ether extract concentration are showed at Table 1.

Table 1. The effects of molasses, lactic acid bacteria (LAB) and LAB and enzyme mixture supplementation on the pH, and chemical composition of sunflower silage

	Treatmentgroups					
Item	Control	Molasses	LAB	LEN	SEM	P
pH	4.26	4.23	4.24	4.21	0.01	NS
Dry matter, %	34.74^{ab}	35.51^{a}	31.89c	33.65b	0.41	**
Crude Protein, % DM	7.94^{a}	7.96^{a}	6.33^{b}	7.27^{a}	0.20	**
Crude cellulose, % DM	21.94^{a}	19.56^{b}	22.90^{a}	21.53^{b}	0.51	*
Crude ash, % DM	13.63^{a}	12.72^{a}	10.05^{b}	13.72^{a}	0.46	**
Ether extract, % DM	4.53	4.35	5.36	4.58	0.20	NS
Neutral detergent fiber, % DM	39.62^{a}	35.70^{b}	42.14^{a}	39.82^{a}	0.73	**
Acid detergent fiber, % DM	34.27^{a}	30.81^{b}	36.41^{a}	34.92^{a}	0.64	**
Hemi-cellulose, % DM	5.35	4.89	5.73	4.9	0.28	NS
Acid detergent lignin, % DM	12.34	11.66	13.51	13.39	0.43	NS

$^{a,b,c:}$ Values with different superscript in a line differ significantly; LAB: Lactic acid bacteria inoculant, LEN: enzyme and bacteria inoculant P:probability; *:P<0.05; **:P<0.01; SEM: pooled standard error of means. NS: non significant

There were no statistically differences between the treatment groups in terms of pH, and ether extract values. Similar findings were observed that molasses addition to ryegrass (Islam et al., 2001) and Lab inoculant addition to corn silage (Filya et al., 2004) did not chance silage pH. Addition of 5% molasses caused an increase in DM content compared to LAB and LEN groups. In the LAB group the DM was lower than those of other groups (P<0.01). Islam et al. (2001) and Gul et al. (2008) noted that molasses addition caused an increase in DM content of grass silages. However, Ozduven et al. (2009) reported that LAB inoculant and LEN addition and Polat et al. (2005) LAB inoculant addition to sunflower silage material did not affect DM content of sunflower silage. Similar findings were reported by Meeske and Basson (1998) and Filya and Sucu (2006) in corn silage LAB inoculant addition did not affect DM of corn silage. In the LAB group crude proteinand ash were lower than those of C, M and LEN groups (P<0.01).In the experiment LAB supplementation may be increased lactic acid bacteria and thus, bacteria used some protein and minerals to bacterial growth and improvement. Ozduven et al. (2009) and Koc et al. (2009) reported that LAB and enzyme mixture inoculants did not change crude protein, ash and ether extract of sunflower silages. The crude cellulose content of 5% M and LENgroups were lower than those of C and LAB groups(P<0.05).In the M group, ADF and NDF values were lower than those of other groups (P<0.01).There were no

significant differences among the groups in terms of HEM and ADL values.Islam et al (2001) determined that molasses and bacterial inoculants reduced cellulose components in grass silages. However, Ozduven et al. (2009), Koc et al. (2009) reported that LAB and enzyme mixture inoculants did not change crude protein, ether extract, crude cellulose, ADF, NDF, ADL, HEM contents of sunflower silages. As a biological material, effectiveness of silage additives and silage fermentation characteristic may vary according to ensiled material properties, ensiling techniques, stored conditions and properties inoculants (Kılıç, 1986; Henderson and McDonald, 1984).

The effects of M, LAB and LEN supplementation on the WSC, lactic, acetic, propionic and butyric acid concentration of treatment groups are showed at Table 2.

Table 2. The effects of molasses, lactic acid bacteria (LAB) inoculants and enzyme supplementation on the water soluble carbohydrate (WSC), lactic, acetic, propionic and butyric acid concentration of sunflower silage

	Treatment groups					
Item	Control	Molasses	LAB	LEN	SEM	P
Water soluble carbohydrate, g/kg DM	2.43^{c}	3.19^{a}	2.50^{c}	2.69^{b}	0.079	**
Lactic acid, % DM	5.51^{a}	5.84^{a}	4.97^{a}	2.87^{b}	0.427	*
Acetic acid, % DM	1.94^{ab}	2.64^{a}	2.71^{a}	0.56^{b}	0.327	*
Propionic acid, % DM	0.03	0.01	0.03	0.06	0.010	NS
Butyric acid, % DM	ND	ND	ND	ND	-	-

LAB: Lactic acid bacterial inoculant, LEN: enzyme and bacterial inoculant; P: probability; ND: Not detected; *:P<0.05; **:P<0.01; SEM: pooled standard error of means, NS: non significant.

Molasses supplementation was increased WSC concentration compared to other groups. Also, in the LEN group's WSC concentration was higher than those of C and LAB groups (P<0.001). It is reported that WSC content of corn (Ozduven et al., (2010) and sunflower silage (Ozduven et al., 2009) increased by the LAB and enzyme inoculants addition. The lactic acid concentration in the LEN group was lower than those of other groups (P<0.05) andthe acetic acid concentration was lower than M and LAB groups (P<0.05), however there was no statistical difference with C group. There was no difference between the treatment groups in terms of propionic acid concentrations (P>0.05). In silage samples, butyric acid was not determined.It is expected that silage additives such as LAB and LAB+enzyme mixtureinoculants can increase WSC and lactic acid and decrease in pH, acetic, propionic and butyric acid ratio in silages. These additives may support to release fermentable sugars to produce more lactic acid in proportion to other products and lowered pH level (Kung et al., 1991). In the current experiment LA concentration did not change in LAB group and lowered with LEN addition. In contrast to these findings,Islam et al. (2001), Filya et al. (2004), Ozduven et al. (2009, 2010), and Koc et al. (2009) reported LAB and enzyme addition reduced LA concentration in silages.

The effects of M, LAB inoculants and LEN supplementation on the TDN, OM, NFC, TC, ME and FP are given at Table 3. In the M group, TDN and NFC values were significantly higher than those of C, LAB and LENgroups (P<0.01 and P<0.05). The LAB supplementation increased the OM, TC and ME values statistically higher than those of C, M and LEN groups (P<0.01 and P<0.05). However, in the LAB group the FP was lower than those of other groups (P<0.01). No significant effect were determined between the C and LEN groups in terms of TDN, OM, NCF, TC, ME and FP.Molasses and LAB inoculants were increased energy value parameters. Islam et al (2001) reported that molasses and inoculant addition did not affect gross energy of silages. Feed value of ensiled feed is affected by variety of factors such as seed content that varies according to vegetation period, NDF content and digestibility, carbohydrate, fat and protein content of feed (Bal et al., 1997).

Table 3. The effects of molasses, lactic acid bacteria (LAB) inoculants and enzyme supplementation on the total digestibility nutrients (TDN), organic matter (OM), non-starch carbohydrate (NFC), total carbohydrate (TC), metabolizable energy (ME) and fleig point (FP)

	Treatment groups					
Item	Control	Molasses	LAB	LEN	SEM	P
Total digestibility nutrients, g/kg DM	78.26[b]	80.93[a]	76.55[b]	78.13 [b]	0.497	**
Organic matter, g/kg DM	86.37[b]	87.28 [b]	89.95[a]	86.28 [b]	0.458	**
Non-starch carbohydrate, g/kg DM	34.28[b]	39.28[a]	36.12[b]	34.61 [b]	0.659	*
Total carbohydrate, g/kg DM	73.90[b]	74.97 [b]	78.26[a]	74.43 [b]	0.584	*
Metabolizable energy, kcal/kg DM	12.34[b]	12.47 [b]	12.69[a]	12.34 [b]	0.044	**
Fleig point	104.18[a]	107.03[a]	99.28[b]	103.80[a]	0.881	**

[a, b,c:] Values with different superscript in a row differ significantly; P:probability; *:P<0.05; **:P<0.01; SEM: pooled standard error of means. LAB: Lactic acid bacteria inoculant, LEN: enzyme and bacteria inoculants.

Conclusion

In conclusion, addition of molasses increased DM and lowered ADF and NDF level in silage samples. Also, molasses inclusion increased WSC and LA concentration in silages. The LEN group (LAB+enzyme mixture) did not influence positively silage quality traits. The molasses and LAB inoculant addition positively affected silage TDN, OM, NFC, TC and ME levels. According to these results molasses and LAB can be preferred as a sunflower silage inoculant.

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INSTALLATION TRANSMITTER ELECTRONICS WITH SURGERY AND DIFFERENT HEALING METHODS ON RAINBOW TROUT (Oncorhynchus mykiss)

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Abstract

In this study, it was aimed to install electronic transmitters (radio tags) with surgical operation without resorting to euthanasia and after the incision to Rainbow trout and to heal by using waterproof tape and disinfectant, antibiotic.

Forty eight rainbow trouts about 250-260 g were used in the surgery operations and then were placed in six tanks with 500 lt volume under laboratory condition after the surgery. The tank water was kept in suitable temperature and dissolved oxygen with a well aeration and continuously cooling. Fishes were anaesthetized with 0,4 ml/L 2- phenoxyethanol by bath method after they were starved for a day. The continuous water containing anesthetic 0.2 ml / L 2-phenoxyethanol were given to the mouths of fishes with a hose. Fish anus to about 7 cm, ventral fins 3 cm, pectoral fins 2 cm, operculum 5 cm, so that the incision 3 cm long; puncture of a sterile scalpel sharp on the full abdominal cavity facing and made an incision by followed were opened muscle and layers subcutaneous connective tissue, skin. Electronic transmitter was inserted into the abdominal cavity. Then of the incision was discarded 3 of the operation sewing needle. The purpose of improve the incision fish was used to heal of penicillin, disinfectants, waterproof tape, disinfectant +waterproof tape and pensile the + waterproof tapes. Complete healing of surgical was observed in 20 days in the control group not given anything; 15 days group at penicillin, in disinfectants and disinfectant + waterproof tape; 10th day of the pensile + waterproof tape group.

This research demonstrated that surgery can be done in the future fish, in particular the internal organs of the biopsy has demonstrated to be contribution to a very important.

Key Words: *Rainbow Trout, Oncorhynchus mykiss, Punction, Incision, Surgical Operation, Electronics transmitter*

Introduction

Use of electronic transminatter (radio tag) in the world has been started from the 17th century, especially in recent years, fishes economically important and endemic is used citations various known electronic tag placed inside the body in order to examine the behavior (Thorsteinsson, 2002; Lucas and Bubb, 2005; Enders et al., 2007; Nelson et al., 2007). In recent years, their habitat use and fish migration with the development of electronic brand technology has become more important (Thorsteinsson, 2002).

The objective of the operation applied to live on anesthesia is to ensure that slowing of the metabolic rate live, not suffering from the intervention of living, facilitating the intervention in living, by slowed down to stop the reflex response, destruction or decreased consciousness, lively sense of recruitment (Çetinkaya and Şahin, 2005).

Surgical applications in fish applied removal of tissue for treatment of nonpathological and other pathologic, removal various organs and tumor, various organ biopsy, sperm and egg sampling, branding, implant placement, drug injection, blood, vaccines (Akyol and Ceylan, 2003).

Studies surgical operation made could not determin morphological characteristics of maturity gender and sex in Sturgeon (Akbulut et al., 2005). But trout or other fish in operation has not been observed in studies.

Recent years to examine individual behavior in fish electronic tag placed into the body fish have been used. Radio tags, also known as transmitter tag used to investigate the more fish migration and habitat (Thorsteinsson, 2002; Anonim, 2015b; Hale and Gray, 1998; Ramstad and Woody, 2003; Brennan et al., 2005; Neely et al., 2009).

After the operation the fish disinfectant and antibiotic water-soluble control dose to prevent infection can be applied (Anonim, 2015a).

People, especially evaluation stages and for follow-up the surgical wound can be an important record about the development of the photographing process after receiving the approval of the patient (Yüceyar, 2008). In a study conducted in fish is made assessment depending on whether or redness and inflammation of the incision looking over the eye clinical examination of the surgical wound (Pedersen and Andersen, 1985).

Successful survival of Rainbow trout has been achieved by inserting an electronic brand (radio tag) through a surgical operation and treating the region with antibiotics, disinfectants and waterproof tape after incision without the need of euthanasia. To our knowledge, this study demonstrates the use of antibiotics, disinfectants and water-proof tapes to heal the incision region of fish for the first time. Additionally, with the results generated here, it has been clearly shown that this research can significantly contribute to surgical interventions, especially internal organ biopsies, which may be performed on fish in the future. Lastly, we believe that the results of this study will be beneficial for the operation studies to be conducted to determine the gender and sexual maturity of Sturgeon fish and to the electronic brand projects that aim at investigating migration routes, migration time, habitat selectivity, breeding areas, and breeding biology of other fish.

Materials and Methods

The study was carried out at the Fish Disease Laboratory, Fisheries, Faculty of Agriculture, Kahramanmaraş Sütçü Imam University (Turkey). Rainbow trout has been used in surgery for 250-300 g in weight at 48 number. The fish tank was placed 500 liters at 6 number. Trout is expected 7 days to ensure adaptation to the environmental conditions. Fish were used 3 mm diameter pellets feed during the study period. Surgical operation was used operating table, forceps, pointed scalpel, scissors, catgut suture material in the needle holder scissors, cotton, alcohol, electronics brand (radio tag) waterproof tape, disinfectant and penicillin powder, plaster and waterproof tape. These materials is made in the laboratory disinfection or sterilization. For the procedure anesthesia to fishes was added 4 ml of 2-phenoxyethanol 10 liters of water into bucket. Fishes is provided inactivity completely in about 1 minutes in anesthetic solution. Fishes are given sustained mouth water and placed supine on the operating table prepared maximum care under aseptic conditions. For lack contamination in operation were sterile instruments to be used. Cross-sectional area has been cleared with the help of cotton excess mucus and disinfected with sterile saline. Fish anus to about 7 cm, ventral fins 3 cm, pectoral fins 2 cm, operculum 5 cm, so that the incision 3 cm long; puncture of a sterile scalpel sharp on the full abdominal cavity facing and made an incision by followed were opened muscle and layers subcutaneous connective tissue, skin. Electronics brands (radio tag) that will be placed have been disinfected with 96% ethyl alcohol. Electronic transmitter (radio tag) was inserted into the abdominal cavity. Then instead of these operations have been taken 1. sewing, 2. sewing and 3. sewing instead of the surgical needle. Operation place was applied waterproof tape, disinfectant and penicillin specified in the group. Groups are described Table 1

Table 1. Experimental groups

1. group	**2. group**	**3. group**	**4. group**	**5. group**	**6. group**
Control	Penicillin	Disinfectant	Waterproof tape	Disinfectants + waterproof tape	Penicillin + waterproof tape
7 number	8 number	8 number	8 number	8 number	8 number

Each group of fish during the experiment was given feed twice including daily morning and evening until regimens. During the experiment, the water in the tank containing the fish were taken into value oxygen, pH and temperature daily.

Results and Discussion

Rainbow trout has been provided successful survival of fish improvement using of antibiotics, disinfectants and waterproof tape after incision and placing electronic brand (radio tag) with surgical operations without resorting to euthanasia.

The most widely used anesthetics in the fisheries industry were MS-222, benzocaine, kinaldin, 2-Fenoksietanol, clove oil, klorbütanol, metomideyt, propanidit, etomideyt. Besides like the ketamine, the saffan, the halato, chloralhydrate, diazepam was used a anesthetic and sedative. But these used relatively less than others (Lemmer, 1993; Kanyılmaz et al., 2007). In this study, 2-Phenoxyethanol was preferred due to the very use in the fishing business and absence of toxic.

Live exposed anesthetic agent occur unresponsiveness after movement and loss of balance, before sedation, depending on the concentration and exposure time and finally disappear reflex action (Çetinkaya and Şahin, 2005). This study fishes were observed similar findings during anesthesia.

During surgical operation fish is performed oxygen enrichment and continuation of anesthesia with water anesthetic mixture by a suitable pipe mouth. The deep is provided the continued operation by controlling the temperature and to ensure remain moist. At the end of operation is expected the recovery in minutes in the post-operation tanks air-conditioned (Çetinkaya and Şahin, 2005; Kanyılmaz et al., 2007; Akyol and Ceylan, 2003). In this study, made of similar application fish has not been any loss during surgery.

Fish skin consists of two layers (Ekingen, 2001; Arda et al., 2005). Epidermis forms the uppermost layer is typically very thin (Demir, 2006). Epidermis is located secreting cells (goblet cells, mucous cells) mucoid substance (protein, glycoprotein, proteoglycan structure in). Therefore, This top skin is very slippery and It is coated with a layer of mucoid (Arda et al., 2005). Typically superficial is up to 250 microns the average thickness, consist of flat cells several times (10-30 times) (Ekingen, 2001). In this study, deep layer thickness has changed based on the size and sex of fish.

This study made by Pedersen and Andersen (1985) was boiled for 30 minutes boiling water to sterile materials to be used in surgical procedures. Also He waited 65% ethyl alcohol sewing thread and the operating table. We used sterile surgical gloves to avoid contamination. In this study, materials used in surgical procedures were sterilized 45 minutes at 121 ° C at 1 atmosphere autoclave. Also operating table has been cleared thoroughly disinfectant odorless, colorless, derived from salt and pure water 100% natural. Immediately before the surgical procedure was heated at ethenol electronics brands (radio tag) and operating table was wiped with 96% alcohol.

Works electronic transminatter (radio tag) in the world has started from the 17th century, especially in recent years, fishes economically important and endemic is used citations various known electronic tag placed inside the body in order to examine the behavior (Thorsteinsson, 2002; Lucas and Bubb, 2005; Enders et al., 2007; Nelson et al., 2007). This

study laboratory was placed in the abdominal cavity the fish with surgical operation electronic tag successfully.
In a study has been reported does not cause death on fish and 25 cm the radio tag (Lucas and Bubb, 2005). In this study there has been death losses arising from any radio tag.
In care and tracking surgical wound in humans is important be registered the condition of the wound bed, swelling, color, smell, infection, pain, condition of the wound edge, regularly to follow daily and using a form. Especially to stages evaluation and followed by open wounds, after receiving the approval of the patient, the photographing process can be an important record of the development. However, taking picture of the wound may contain problems according structure socio-cultural to the patient's (Yüceyar, 2008),. In this study is taken photograph followed by performing daily in a days 5th 10th 15th and created a form as in the maintenance and tracking of surgical wounds in humans (Table 2-3-4-4).
According to Pedersen and Andersen (1985) from surgical operation has observed inflammation and redness over incision 5-7 days. Inflammation disappeared decrease in 4-8 days. Location of the incision is completely healed 24-34 days. In this study It was observed in waterproof band group and control 5th days only inflammation and erythema. Location of the incision was observed complete recovery 15th day with penicillin, disinfectant, disinfectant + waterproof tape; 10th days without any contamination penicillin + with waterproof tape. However, in the control group not applied anything were observed redness and inflammation 5th day. Completely healing recorded 20th day in the control group not implemented anything; 15 days in group penicillin, disinfectant and disinfectant + waterproof tape; 10th days the group penicillin + waterproof tape
Before starting surgical operation fishes, antibiotic can provide against infection using a protective dose. At the end of the operation recommended to continue to practice antibiotic 1-4 weeks (ScottWeber et al., 2009). In this study is used to close location incision immediately after surgery antibiotics (penicillin) to be contaminated at the surgical site.

Table 2. Form evaluation and follow 5th day after surgical operation.

	Experimental groups					
	Control group	Penicillin	Disinfectant	Waterproof tape	Disinfectants + waterproof tape	Penicillin + waterproof tape
Number of fish	7	8	8	8	8	8
The number of fish the dead	-	-	-	-	-	-
Status receive feed (% 3)	1/3	3/3	3/3	3/3	3/3	3/3
Motion Status	Slow	Normal	Normal	Slow	Normal	Normal
Status fusion incision edge	No	There are some places	There are some places	No	There are some places	Yes
Reduction of sewing thread	-	-	-	-	-	-
Infection	-	-	-	-	-	-
Inflammation	+	-	-	+	-	-
Smell	-	-	-	-	-	-
Erythema	+	-	-	+	-	-
Swelling	-	-	-	-	-	-
Photo shoot						

Table 3. Form evaluation and follow 10th day after surgical operation.

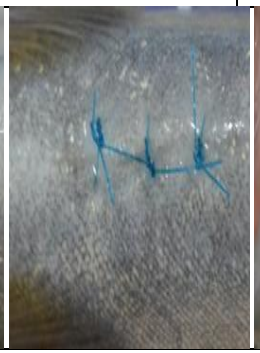
	Experimental groups					
	Control group	Penicillin	Disinfectant	Waterproof tape	Disinfectants + waterproof tape	Penicillin + waterproof tape
Number of fish	7	8	8	8	8	8
The number of fish the dead	1	-	-	1	-	-
Status receive feed (% 3)	2/3	3/3	3/3	3/3	3/3	3/3
Motion Status	Slow	Normal	Normal	Slow	Normal	Normal
Status fusion incision edge	There are some places	Yes	Yes	There are some places	Yes	Complete recovery
Reduction of sewing thread	-	1	-	1	-	1
Infection	-	-	-	-	-	-
Inflammation	+	-	-	-	-	-
Smell	-	-	-	-	-	-
Erythema	+	-	-	-	-	-
Swelling	-	-	-	-	-	-
Photo shoot						

Table 4. Form evaluation and follow 15th day after surgical operation.

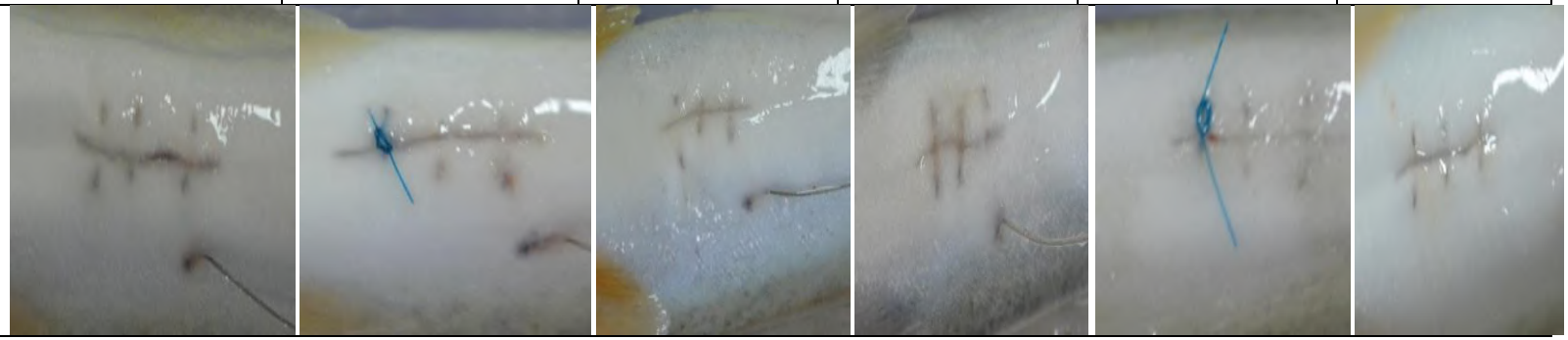
	Experimental groups					
	Control group	Penicillin	Disinfectant	Waterproof tape	Disinfectants + waterproof tape	Penicillin + waterproof tape
Number of fish	7	8	8	8	8	8
The number of fish the dead	-	-	1	-	1	-
Status receive feed (% 3)	3/3	3/3	3/3	3/3	3/3	3/3
Motion Status	normal	normal	normal	normal	normal	fast
Status fusion incision edge	yes	yes	yes	yes	yes	yes
Reduction of sewing thread	3	2	3	3	2	3
Infection	-	-	-	-	-	-
Inflammation	-	-	-	-	-	-
Smell	-	-	-	-	-	-
Erythema	-	-	-	-	-	-
Swelling	-	-	-	-	-	-
Photo shoot						

Table 5. Form evaluation and follow 20th day after surgical operation.

	Experimental groups					
	Control group	Penicillin	Disinfectant	Waterproof tape	Disinfectants + waterproof tape	Penicillin + waterproof tape
Number of fish	7	8	8	8	8	8
The number of fish the dead	-	-	1	-	1	-
Status receive feed (% 3)	3/3	3/3	3/3	3/3	3/3	3/3
Motion Status	Normal	Hızlı	Normal	Normal	Normal	Hızlı
Status fusion incision edge	Yes	Yes	Yes	Yes	Yes	Yes
Reduction of sewing thread	3	2	3	3	2	3
Infection	-	-	-	-	-	-
Inflammation	-	-	-	-	-	-
Smell	-	-	-	-	-	-
Erythema	-	-	-	-	-	-
Swelling	-	-	-	-	-	-

Photo shoot	

Conclusion

First time in Turkey, rainbow trout has been finalized successfully placing electronics brands (radio tag) and applied to such an operation technique.

In human and veterinary medicine has been very difficult really apply in fish such a study looks very simple. So far fishes haven't been made such a surgical intervention. With this study; If rainbow trout implemented necessary of rules antisepsis after the operation, they could stay successfully live; fishes can be done in surgical intervention the future; especially In enzymatic studies with liver biopsy; the examination of histological structure of the liver and It has been put forward may be of a very important contribution to studies as ultrasound taking of cystic structure in the presence of internal organs.

In improving the incision of fish has been used for the first time successfully antibiotics, disinfectants and waterproof tape

Rainbow trout has been provided successful survival of fish after incision improvement using of antibiotics, disinfectants and waterproof tape and electronic brand (radio tag) placing with surgical operations without resorting to euthanasia. This research, fish in the surgery can be done in the future, in particular, it has put forward would be very significant contribution to the internal organs biopsies. Furthermore, the fish are used to improve the location of the incision the first antibiotic, disinfectant and waterproof tapes. These study results, Sturgeon will make a significant contribution penicillin + waterproof improving soon 10 days place the surgical operation the study in electronics brand such as other fish migration routes, migration time, habitat selectivity, breeding areas, breeding biology and operating activities used to determine sex and sexual maturity.

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THE EFFECT OF THE ORDER OF LACTATION ON REPRODUCTIVE CHARACTERISTICS OF SIMMENTAL COWS

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Abstract

Cattle farming is the leading branch of livestock production in almost all countries of the world. In livestock production it is by far in the first place, despite the constant increase in production of pork and poultry in the world. The aim of this paper is the influence of lactation on reproductive characteristics (age of cows at first insemination, gestation length, service period duration and inter-calving interval duration) in Simmental cows reared on the farm "Mountain Good Nevesinje". The farm is located on 4 locations (Morine, Sopilja, Kovačice and Pomakovci). Based on the data obtained from the population register (selection agency) and monitored reproductive characteristics (age of cow - heifers at first insemination, gestation duration, service period and inter-calving interval duration) in the first three calvings, ie. three lactations, what follows can be concluded: the average age of a cow (heifer) at first conception was 16.84 months, and all were successfully inseminated in 2011. Duration of gestation, depending on the order of the calf, ranged between 288.20 days (first calf) and 287.20 days (second calf). The average service period, or inter-calving interval for the first three lactations (three calvings) amounted to 136.64 days and 423.44 days. Minimum duration of service period, and therefore inter-calving interval was in the third lactation and amounted to 136.64 days, or 397.20 days.

Key words: *lactation, reproductive characteristics of cows and Simmental cattle*

Introduction

The advantage of cattle breeding in comparison with other branches of livestock production is the use of available reserves of forage, namely those that are produced in the fields and meadows, as well as those used by going out on pastures. The base of production is keeping or breeding of cattle and as its main products we have milk and calves, and therefore the production of beef. Yield and quality (composition) of milk are partly hereditary characteristics, ie. they depend on the breed (genotype) of cows. (Mitić et al, 1987); Looper, 2012). Khan et al. (1996) point out that cows age during the calving are affected by the age of cows, especially young cows in early lactation. Reproductive efficiency is the main factor that determines the success of production and economic efficiency in dairy herds (Diskin, 2008). Reproduction of cattle is one of the most important phases of livestock production, because at that stage the heads which are going to replace the herd are provided, as well as for milk production and fattening, and meat production. Many years of intensive selection of dairy cattle breed focused on high milk production or milk production, has significantly influenced the reduction of their fertility, and reproductive efficiency (Rodriguez-Martinez et al., 2008). The main objective of this study was the influence of lactation on reproductive characteristics in Simmental cows reared on the "Planinsko Dobro Nevesinje" farm (age of cow at first insemination, gestation length, service period and inter-calving interval duration).

Materials and methods

By the method of randomly selected sample of 15 cows in first three lactations (a total of 45 cows) the following indicators were observed (age of cows at first insemination, gestation length, service period and inter-calving interval duration). Each cow was identified, ie. had its pedigree with the registration number, date of birth, order of lactation, date of insemination, gestation length, service period and inter-calving interval. Observations were carried out on the farm "Planinsko Dobro Nevesinje", which, among other things, deals with the breeding and cultivation of dairy cows or production of milk. The basic data processing and testing of the significance of differences between the tested parameters was performed using conventional variation - statistical methods and analysis of variance (Hadživuković, 1991), and calculations of the coefficients of phenotypic correlation connection between the established parameters was performed according to the model for the appropriate sample (Latinović, 1996).

Results and discussion

According to the textbook literature, cows (heifers), depending on the race, reach sexual maturity between 11 and 15 months. They are sexually active throughout the year, and the gestation lasts between 278 and 288 days, although some minor deviations that are caused by abiotic factors are possible. Accordingly, in Tables 1 and 2, the results of our tests are determined in terms of age of cows (heifers) at their first conception, gestation duration, inter-calving interval, and service period duration for the I, II and III lactation, ie. calving of cows. A series of genetic and non-genetic factors affect the service period: cattle breed, the amount of daily milk production, the parity of calving, nutrition and housing, health condition of cows on the farm, the state of the reproductive organs of cows, estrus detection, timely insemination, etc. (Orešnik, 2009) .

Table 1. Average values and variability of age of cows, inter-calving period and service period in the first three lactations

(n – 15 + 15 + 15 = 45)

Lactation	**Indicators**	$\bar{x}$	$S_{\bar{x}}$	**S**	**C.V. (%)**
I	Age of cows – first insemination (months)	17,87	0,55	2,13	11,92
	Inter-calving period (days)	437,07	10,32	39,99	9,15
	Service period (days)	148,87	6,13	23,76	15,96
II	Age of cows – first insemination (months)	16,20	0,28	1,09	6,73
	Inter-calving period (days)	436,07	18,50	71,64	16,43
	Service period (days)	151,07	7,95	30,81	20,30
III	Age of cows – first insemination (months)	16,44	0,49	1,90	11,58
	Inter-calving period (days)	397,20	9,85	38,15	9,60
	Service period (days)	110,00	9,62	37,27	9,62
I+II+III	***Age of cows – first insemination (months)***	***16,84***	***0,27***	***1,91***	***11,34***
	Inter-calving period (days)	***423,44***	***8,02***	***53,78***	***12,70***
	Service period (days)	***136,64***	***53,60***	***35,95***	***26,31***

The data in Table 1 show that the average age of the three groups of cows at first successful insemination was 16.84 months, respectively 17.87 months (group of cows that is monitored during the first lactation - first calving), 16.20 months (cows during the second lactation – the

second calving) and 16.44 months (third lactation cows - the third calving). The differences in terms of age of cows at first insemination were not statistically significant (P> 0.05).

Table 2. Average values and the variability of the duration of gestation depending on the age of the cow and gender of the calf (days)

Gestation	Calf gender	n	$\bar{x}$	$S_{\bar{x}}$	S	C.V. (%)
I	♂	9	288,11	0,77	2,15	0,75
	♀	6	288,33	0,66	1,63	0,56
	♂ + ♀	15	288,20	0,49	1,90	0,66
II	♂	8	287,25	0,45	1,28	0,45
	♀	7	287,14	0,59	1,57	0,55
	♂ + ♀	15	287,20	0,35	1,37	0,48
III	♂	6	288,50	0,56	1,38	0,48
	♀	9	287,67	0,60	1,80	0,63
	♂ + ♀	15	288,00	0,43	1,65	0,57
I+II+III	♂	***23***	***287,91***	***0,35***	***1,70***	***0,59***
	♀	***22***	***287,68***	***0,36***	***1,67***	***0,58***
	♂ + ♀	***45***	***287,80***	***0,25***	***1,65***	***0,57***

The duration of gestation, regardless of the order of lactation calf gender was approximately the same, and in average the duration of gestation in the first three lactation periods for both genders was 287.80 days (Table 2). In addition, absolute and relative measures of variability in terms of the duration of gestation were very small (CV - less than 1%), and the determined differences between the duration of gestation (Table 3) were not statistically significant (P> 0.05).

Table 3. The significance of differences in age of cows, gestation, inter-caving interval duration and service period duration in the first three lactations
(n - 15 + 15 + 15 = 45)

Indicators	Lactation	Average	Difference	Significance
Age of cows, months	I-II	17,87-16,20	1,67	Ns
	I-III	17,87-16,44	1,43	ns
	II-III	16,20-16,44	-0,24	ns
Duration of gestation, days	I-II	288,20-287,20	1.00	Ns
	I-III	288,20-288,00	0,20	ns
	II- III	287,20-288,00	-0,80	ns
Inter-calving interval, days	I-II	437,07-436,07	1,00	Ns
	I-III	437,07-397,20	39,87	**
	II-III	436,07-397,20	38,87	*
Service period, days	I-II	148,87-151,07	-2,20	Ns
	I-III	148,87-110,00	38,87	**
	II-III	151,07-110,00	41,07	***

***P<0,001; **P<0,01; *P<0,05; ^{nc}P>0,05

In contrast to the duration of gestation of cows, the duration of inter-calving interval, and service period in particular showed large variability, both in absolute (S) and in a relative (C.V.%) measures of variability (Table 1). In general (the first three lactations), the coefficient of variation was 38.92% (service period duration) and 12.70% (inter-calving period).

Conclusion

Based on the data from the system records (selection service) and monitoring of the reproductive characteristics (age of cow - heifers at first insemination, gestation duration, service period and inter-calving interval duration) during the first three calvings ie. three lactations, the following conclusions can be derived:

- The average age of the cows (heifers) at first conception was 16.84 months, and all were successfully inseminated in 2011. Duration of pregnancy, depending on the order of the calf, ranged between 288.20 days (first calf) and 287.20 days (second calf).
- The average service period duration, or inter-calving intervals duration for the first three lactations (three calvings) amounted to 136.64 days and 423.44 days. The minimum duration of service period, and therefore inter-calving interval during the third lactation and the amount is 136.64 days, or 397.20 days.

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THE EFFECT OF THE ORDER OF LACTATION ON PRODUCTION CHARACTERISTICS OF SIMMENTAL COWS

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Abstract

The aim of this study is the effect of the order of lactation on production characteristics (milk yield, yield and fat content in milk, yield and protein content) and determination of phenotypic correlation connection (r_p) between production characteristics, and statistical significance of the difference in the first three lactations of Simmental cows grown on the farm "Planinsko Dobro Nevesinje". Duration of the first lactation was the longest (372.07 days) and the shortest was (321,80 days) in the third production lactation, averaging 347.69 days (for all three lactations). The duration of production lactation is directly reflected on the yield of milk, which in the first lactation was 8019.73 kg, 7864.87 kg in the second, and 7341.40 kg in the third. Although the first lactation was the longest, and the third was the shortest, the daily production per cow was the highest in the third lactation (23.02 kg), followed by the second lactation (22.87 kg) and the lowest production was in the first lactation (21.70 kg), ie. with the aging of the cows the production of milk increased. In relation to production lactation (whole), milk yield, calculated in standard lactation (305 days), was averaging at 7,272.93 kg. Between milk yield throughout the whole lactation and milk yield in standard lactation there is a strong positive correlation connection. With increasing milk yield in standard lactation, protein content, and especially milk fat content decreased.

Key words: lactation, production characteristics, Simmental cows.

Introduction

In livestock production, milk is the most important product, while in a human consumption it is one of the most important foods. In developed countries the average daily consumption of milk per capita is 1 liter (Tošić et al., 2002). In addition, a significant number of non-genetic factors affects the milk yield (the effect of lactation on milk production of cows and milk composition, the effect of the order of lactation on cow milk production and milk composition, milking influence, the influence of estrus, breeding and health, the impact of nutrition during breeding on future milk production, the impact of nutrition during lactation, the impact of environmental conditions on cow milk production and milk composition, water content, fat centent, protein content, lactose and mineral substances in milk during lactation (Mitić et al., 1987; Looper, 2012). The effect of lactation on yield, milk fat yield and milk fat content, protein yield and protein content in milk was examined by many authors (Nenadović, 1974; Uremović 1980, Panić et al., 1983; Skalicki, 1983).

The main objective of this study was to evaluate the production characteristics (milk yield, milk fat yield and milk fat content, protein yield and protein content) that affect lactation.

Materials and methods

The tests were conducted on the farm "Planinsko Dobro Nevesinje", which, among other things, deals with the breeding and cultivation of dairy cows, and milk production. On this farm, if it is operating at its full capacity, milk production can be organized with 543 dairy cows and related categories of cows. Simmental breed of cattle imported from Czech Republic are used for the production of milk (and meat). By the method of random selection, 15 cows in the first three lactations (a total of 45 cows) were selected and the following parameters were observed: milk yield, milk fat yield and milk fat content, protein yield and protein content. The basic data processing and testing the significance of differences between the investigated parameters was performed using conventional variation - statistical methods and the analysis of variance (Hadživuković, 1991), and the calculating of the coefficients of phenotypic correlation connection between the established parameters was performed according to the model for the appropriate sample (Latinović, 1996).

Results and Discussion

In the analysis of the data the indicators of productivity of cows are presented in the table, ie. milk production (production and standard lactation), and milk yield (kg), yield (kg) and the content of milk fat (%) and proteins for the first three lactations of dairy cows which were analyzed.

Table 1. Average values and variability of the duration of lactation and milk production (n - 15 + 15 + 15 = 45)

Lactation	**Indicators**	$\bar{x}$	$S_{\bar{x}}$	S	C.V. (%)
I	Lactation duration (days)	372,07	8,88	34,40	9,25
	Daily milk production, per cow, kg	21,70	0,51	1,99	9,17
	Milk yield (PL), kg	8.019,73	103,19	399,66	4,98
	Milk yield (SL), kg	7.300,00	121,49	470,52	6,44
II	Lactation duration (days)	349,20	16,75	64,88	18,58
	Daily milk production, per cow, kg	22,87	0,55	2,13	9,31
	Milk yield (PL), kg	7.864,87	207.84	804,96	10,23
	Milk yield (SL), kg	7.373,67	82,87	320,96	4,35
III	Lactation duration (days)	321,80	13,79	53,40	16,59
	Daily milk production, per cow, kg	23,02	0,50	1,94	8,43
	Milk yield (PL), kg	7.341,40	116,94	452,92	6,17
	Milk yield (SL), kg	7.145,13	120,59	467,03	6,54
I+II+III	***Lactation duration (days)***	***347,69***	***8,18***	***54,86***	***15,78***
	Daily milk production, per cow, kg	***22,53***	***0,31***	***2,05***	***9,09***
	Milk yield (PL), kg	***7.742,00***	***111,42***	***747,40***	***9,65***
	Milk yield (SL), kg	***7.272,93***	***64,96***	***435,79***	***5,99***

In Table 1, we see that the duration of the first lactation period was 372.07 days, for the second it was 349,20 days, and for the third it was 321,80 days, averaging 347.69 days for all three lactations. In addition to the fact that all three lactations lasted much longer than the standard lactation duration (305 days), the variability between cows was high, especially in the second lactation, C.V. - 18.58%. With the aging of cows, the duration of lactation was

shortened, approaching the standard lactation, and daily milk production increased. The highest daily milk production per cow was in the third lactation (23.02 kg), and lowest was in the first (21.70 kg), while the average daily production for all three lactations was 22.53 kg (Table 8).

Analogously, the milk yield in the longest production lactation (whole) was the largest and vice versa. In the first lactation production average milk yield per cow was the highest (8019.73 kg), and the lowest was in the third lactation (7341.40 kg), averaging 7742.00 kg for all three of lactations. Converted to the standard lactation (305 days), milk yield per cow was the largest in the second lactation (7,373.67 kg), slightly lower in the first (7,300.00 kg) and the lowest in the third lactation (7145.13 kg), averaging 7272.93 kg of milk.

Table 2. The significance of the difference in the duration of lactation, daily milk production, yield and content of milk fat and protein

(n - 15 + 15 + 15 = 45)

Indicators	**Lactation**	**Average**	**Difference**	**Significance**
Lactation duration (days)	I-II	372,07-349,20	22,87	ns
	I-III	372,07-321,80	50,27	**
	II-III	349,20-321,80	27,40	*
Daily milk produstion, per cow, kg	I-II	21,70-22,87	-1,17	ns
	I-III	21,70-23,02	-1,32	*
	II-III	22,87-23,02	-0,15	Ns
Milk yield (PL), kg	I-II	8019,73-7864,87	154,86	ns
	I-III	8019,73-7341,40	678,33	***
	II-III	7864,87-7341,40	523,47	*
Milk yield (SL), kg	I-II	7300,00-7373,67	-73,67	ns
	I-III	7300,00-7145,13	154,87	ns
	II-III	7373,67-7145,13	228,54	ns
Milk fat yield, kg	I-II	313,44-309,69	3,75	ns
	I-III	313,44-288,13	25,31	*
	II-III	309,69-288,13	21,56	Ns
Milk fat content, %	I-II	3,92-3,94	-0,02	ns
	I-III	3,92-3,93	-0,01	ns
	II-III	3,94-3,93	0,01	ns
Protein yield, kg	I-II	291,64-286,24	5,40	ns
	I-III	291,64-266,11	25,53	**
	II-III	284,24-266,11	20,13	Ns
Protein content, %	I-II	3,64-3,64	0,00	-
	I-III	3,64-3,62	0,02	ns
	II-III	3,64-3,62	0,02	ns

***P<0,001; **P<0,01; *P<0,05; ^{ns}P>0,05.

M.Y. (P.L.) = milk yield (production lactation), kg; M.Y. (S.L.) = milk yield (standard lactation), kg; L.D. = lactation duration, days; M.F.Y. = milk fat yield, kg; M.F.C. = milk fat content, %. P.Y. = protein yield, kg; P.C. = protein content, %.

In the preceding section it was said that the duration of the first lactation was the longest (372.07 days) and the shortest (321,80 days) was in the third lactation production. The results show that the established differences between the first and third lactation (50.27 days), and between second and third lactation (27.40 days) were statistically confirmed at the level of P <0.01 and P <0.05. The difference found between the duration of the first and second lactation production (22.87 days) were not statistically significant (P> 0.05).

In terms of average daily milk production the only thing that was statistically significant (P <0.05) was observed difference (-1.32 kg) between the first and third lactation. The observed

difference between the first and second (-1.17 kg), as well as the second and third lactation (-0.15 kg) were not statistically significant (P> 0.05).
Duration of production lactation is directly reflected in the average milk yield and the significance of difference confirmed by data presented in Table 2. The difference found between the first and second lactation (154.86 kg) was not statistically significant (P> 0.05), while the difference between the first and third (678.33 kg), and the second and third lactation respectively (523.47 kg) was statistically significant and was confirmed at the level of P <0.001 and P <0.05.

Table 3. Coefficients of phenotypic correlation between duration of lactation, milk yield, milk fat yield and fat content, protein yield and protein content

(N = 45)

Indicators	M.Y. (P.L.)	L.D.	M.F.Y.	M.F.C.	P.Y.	P.C.
M.Y. (P.L.)	1,000	0,840***	0,948***	0,140ns	0,955***	0,040ns
L.D.		1,000	0,803***	-0,046ns	0,841***	0,072ns
M.F.Y.			1,000	-0,062ns	0,950***	0,093ns
M.F.C.				1,000	-0,086ns	0,455**
P.Y.					1,000	0,111ns
P.C.						1,000
M.Y. (S.L.)	0,634***	-	0,598***	-0,163ns	0,633***	-0,001ns

***P<0,001; **P<0,01; HCP>0,05.

M.Y. (P.L.) = milk yield (production lactation – whole lactation), kg; M.Y. (S.L.) = milk yield (standard lactation), kg; L.D. = lactation duration, days; M.F.Y. = milk fat yield, kg; M.F.C. = milk fat content, %. P.Y. = protein yield, kg; P.C. = protein content, %.

The presented data shows that between milk yield in the production lactation and milk yield in standard lactation there is a strong positive correlation connection and that the determined correlation coefficient (rp = 0.634) was statistically confirmed at the level of P <0.001. Very strong positive correlation connection is found between milk yield and the duration of production lactation (whole) (rp = 0.840), and the determined coefficient was confirmed at the level of P <0.001.
The full positive correlation connection was found between milk yield (production lactation), the yield of milk fat (rp = 0.948 ***), ie protein yield (rp = 0.955 ***), and between the yield of milk (standard lactation), the yield of milk fat (rp = 0.598 ***), and strong correlation connection was found in the yield of protein (rp = 0.633 ***). Between the yield of milk (whole lactation), fat content and protein content, as well as between the yield of milk (standard lactation), fat content and protein content there was found a very weak correlation connection, in fact, there was not even a positive (whole lactation) or negative (standard lactation) correlation connection, and determined coefficients were not statistically significant (P> 0.05).
Between the duration of production lactation, milk fat yield, protein yield a statistically significant value of correlation coefficients was found (P <0.001), rp = 0.803 (M.F.Y.) and rp = 0.841 (P.C.), ie. there is a very strong correlation connection. The duration of lactation did not affect the fat content and protein content, and the value of correlation coefficients stood at -0.046 (M.F.C.), ie. 0,072 (P.C.), and are not statistically confirmed (P> 0.05).
Between M.F.Y. and P.Y. the absolute positive correlation connection (rp = 0.950 ***) was revealed, between M.F.Y and M.F.C. (rp = -0,062ns) or P.C. (rp = 0,093ns) there was not statistically significant correlation connection (P> 0.05), as well as between M.F.C. and P.Y. (rp = 0,086ns), while between M.F.C. and P.C. a statistically highly significant positive correlation was found (P <0.01), rp = 0.455. Between P.Y. and P.C. there is a very weak

positive correlation, and identified coefficient of correlation (rp = 0.111) was not statistically confirmed (P> 0.05).

Conclusion

From what we have stated above, it follows that in the examined cows, grown on the "Planinsko Dobro Nevesinje" farm, the yield and the quality of milk in the first three lactations were at a satisfactory (solid) level. In accordance to that and in accordance with the breeding program of the said farms there is a need to implement appropriate selection measures, ie. on the basis of individual assessment of production and reproductive characteristics, to extract, if necessary, a certain number of cows from the production and perform 'overhaul' of the herd.

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MORPHOLOGICAL AND HISTOLOGICAL FEATURES OF TESTES IN LANDES GANDERS

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Abstract

Morphometric and histological examination of testes in Landes ganders was performed at one-, two- and seven years of age. The testicular morphometry included the following parameters – weight, length and width, diameter of seminiferous tubules (ST), ratio between ST volume and interstitial connective tissue volume. During the breeding season (from the end of December to the end of May) at the studied ages, the weight and size of testes increased greatly. In all investigated males the left testicle was significantly bigger. During the non-breeding season the testicular parenchyma regressed and testes weight decreased too. Contrary to our preliminary expectations significant differences in testicular development depending on age - between one- and two-years old on one hand and seven years old on the other were not found.

Key words: *Landes goose, gander, testes, morphology, histology*

Introduction

The changes in testicular tissue of birds are determined by two primary factors – the age (Bochukov et al., 1995; Gerzilov et al., 2015) and the season (Mori and George, 1978; Deviche et al., 2011; Leska et al, 2012; Islam et al., 2013). In many birds, testes undergo dramatic annual changes in size and, as such, are among the most anatomically and physiologically plastic organs found in adult vertebrates (Deviche et al., 2011). The seasonal dynamics of morphological testicular changes in ganders consists in restoration of spermatogenesis at the beginning of the breeding season, followed by a period of normal spermatogenesis until occurrence of the spermatogenous tissue regression during the non-breeding season (Mori and George, 1978; Leska et al., 2012). In some male birds, including ganders, genetically encoded restoration of reproduction activity is efficient enough to provoke a moderate elevation of testosterone concentrations during the autumn, resulting in substantial development of gonads and sexual behaviour (Leska et al., 2012)

The present study aimed to investigate the anatomical and histological status of testes in Landes ganders related to birds' age and breeding season.

Material and methods

Landes ganders hatched at the same time were used for morphological and histological analysis of testes as followed: 3 ganders during the first breeding season aged 10 months (slaughter in April); 3 ganders after finishing the first breeding season aged 12 months (slaughter in June), 3 ganders during the second season aged 22 months (slaughter in April next year) and one 7-year-old gander slaughtered during the breeding season (in April). The birds were reared in free-range family farm with free access to pasture. Before being slaughtered, the live weight (kg) of birds was individually determined. After dissection of carcasses, testes were removed and the following morphometric measurements were done before the histological processing: weight (g), length, and width (mm). Weight studies were performed by means of analytical OHAUS 2000 scales with precision of 0.01 g (fig. 1).

Fig. 1. Weighing testicles
(Д) – right testis; (Л) – left testis

The length and width of testes were measured with a Vernier caliper with precision of 0.1 mm. Pieces from the equatorial part of the testicular parenchyma, 0.5×0.5×0.5 cm of size were collected for histological and morphometric examinations. Specimens were fixed in fresh Bouin's solition for 72 hours, then submitted to routine procedures: removal of the fixative in ascending alcohol series, clearing with xylene, embedding in paraffin, cutting on a Reichert microtome, mounting of sections 6–7 μm thick on glass slides and staining with haematoxylin-eosin. Examinations were performed with Jenaval light microscope, and findings were documented with a CETI camera. The diameter of seminiferous tubules (μm) was determined by measurements of approximately 30 tubular transverse sections using a standardised eyepiece micrometer. The relative volume (%) of seminiferous tubules and interstitial tissue were determined by the method of Pakenas (1968) on the basis of 30 measurements per testis, using an ocular grid with four hairs of different length, located at specific intervals over the grid circle. The morphometric measurements of testes are presented as mean±SEM.

Results and discussion

During the first breeding season, an active spermatogenesis was observed in seminiferous tubules. All generations of germ cells – spermatogonia, spermatocytes, prespermatids, spermatids and spermatozoa (Fig. 2) were present. The interstitial tissues appeared as thin bands, containing relatively large Leydig cells. During the non-breeding season, testes underwent changes in both seminiferous tubules and in the interstitial connective tissue. The curvature of seminiferous tubules was reduced their lumen has become cleft-like with reduced presence of most germ cell generations except for spermatogonia. In some tubules, detritus cell masses were found out. The interstitial tissue occupied a considerable part of the testicular parenchyma and a variety of cells were present within (Fig. 3). During the second breeding season, the observed histological features were almost the same as during the first breeding season. The artefacts observed in seminiferous tubules reflected the different stages of occurring spermatogenesis. Spermatozoa were attached to the Sertoli cells and formed characteristic groups to the lumen, resembling wheat ears. In the interstitial connective tissue, relatively large Leydig cells could be observed (Fig. 4). The histological picture in testes of the 7-year-old gander was similar to that observed in for the previous studied age (Fig. 5). The results from the weight and macromorphometric studies of testes are presented in Table 1. The weight of the left testis exceeded almost twice that of the right one for all ages and seasons. There was not, however, a similar relationship for the length and width of these organs. Testicular weight during the first breeding season exceeded considerably the weight during the non-breeding season. The values of the gonadosomatic index were higher for all ages during the period of reproductive activities. The data obtained from the measurement of seminiferous tubules' diameters are presented in Fig. 6. It shows that largest diameters were those during the second breeding season, i.e. at 2 years of age. In all ganders during the breeding season, seminiferous tubular diameters exceeded 4–4.5 times the respective values during the non-breeding season. The ratios of seminiferous tubules/intesrtitial connective tissue volumes demonstrated that seminiferous tubules' volume in ganders was the greater during the second breeding season. During the non-breeding season, the proportion of the interstitial tissue was larger – 54.20:45.80 (Fig. 7).

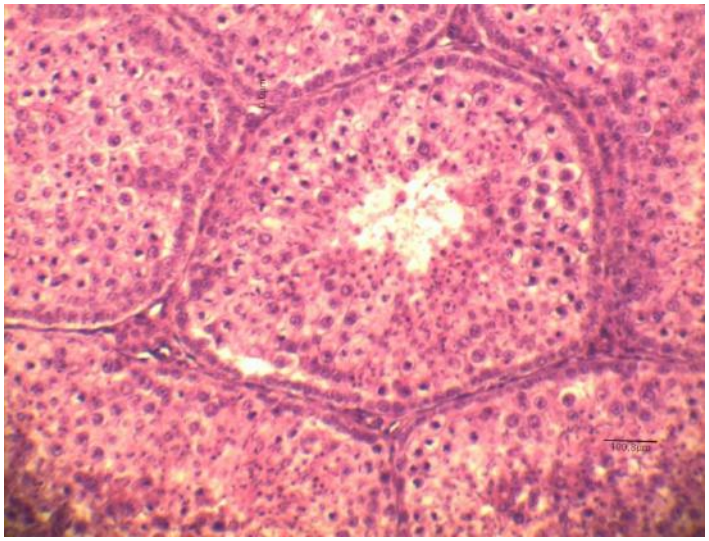

Fig. 2. A gander’s testis during the first breeding season. H/E

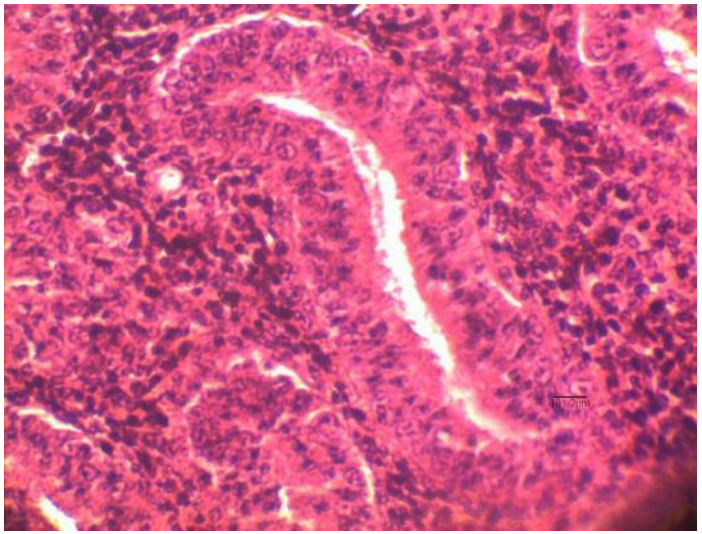

Fig. 3. A gander’s testis during the non-breeding season. H/E

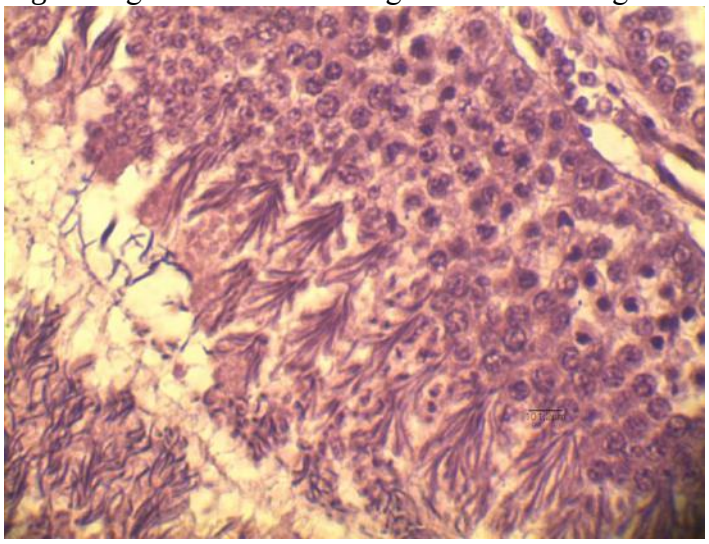

Fig. 4. A gander’s testis during the second breeding season. H/E.

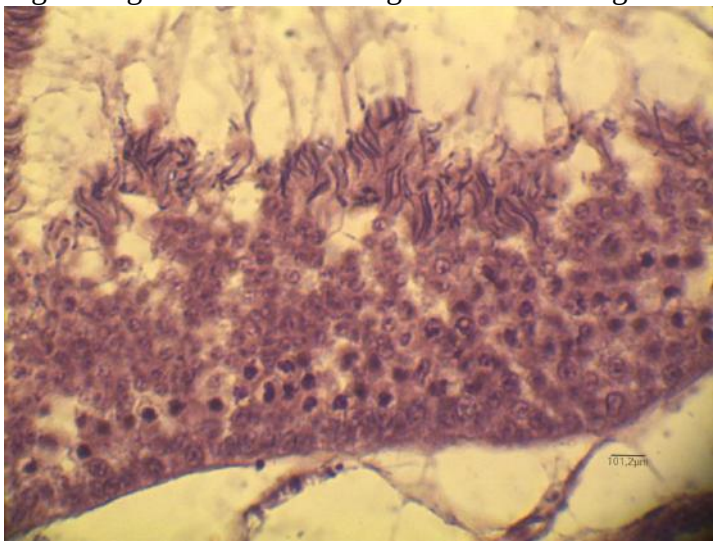

Fig. 5. A 7-year-old gander’s testis. H/E.

Table 1. Macromorphometric dimensions of testes

Age	n	Body weight, g	Testis	Weight, g	Length, mm	Width, mm		Testes weight / Body weight, %
						Dorso-abdominal	Lateral	
First breeding season	3	4617±256	L R	7.33±2.61 4.17±1.64	30.13±3.15 25.40±2.82	20.57±2.66 17.07±3.11	19.17±2.97 14.67±2.12	0.25±0.09
Non-breeding season	3	4900±141	L R	0.35±0.07 0.21±0.04	12.75±0.35 9.93±0.11	7.65±0.21 6.95±0.21	5.90±0.28 6.00±0.14	0.011±0.003
Second breeding season	3	5000±308	L R	12.07±0.47 3.65±0.48	34.00±0.14 21.70±0.32	26.40±0.68 17.63±0.94	24.47±0.39 15.30±0.67	0.32±0.02
7-year-old male in the breeding season	1	6800	L R	8.81 4.06	31.7 23.40	27.4 18.60	19.4 14.90	0.19

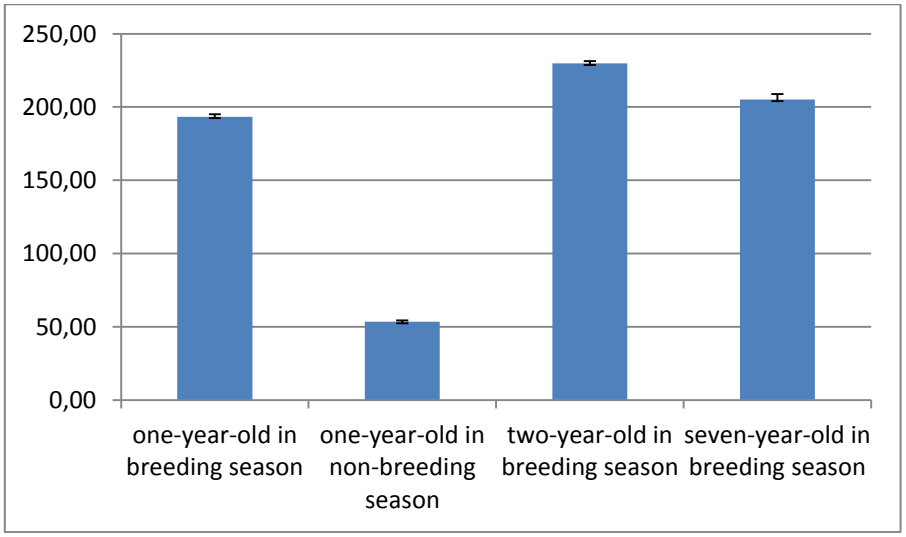

Fig. 6. Seminiferous tubules' diameter (µm) in ganders at a different age and during different seasons.

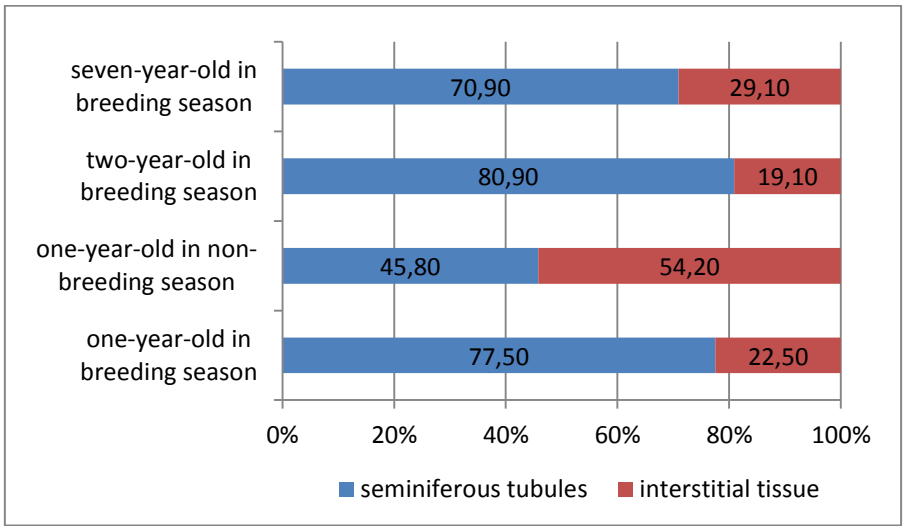

Fig. 7. Seminiferous tubules to interstitial connective tissue ratios

In the present study, signs of actively occurring spermatogenesis were observed in all studied birds during the breeding season. However, we did not found out clearly visible septa, in agreement with the findings of other authors (Bowles, 2014). The presence of anastomoses among seminiferous tubules, which is specific for birds according to Bowles (2014), was not clearly identified. Our results confirmed the presence of cyclic changes in testes in line with other reports (Mori and George, 1978; Lin and Jones, 1990; Deviche et al., 2011; Leska et al., 2012). During the breeding season, all germ cell generations were observed in seminiferous tubules. This specific feature reflecting the different stages of spermatogenesis in birds was described long ago (Johnson, 1961). Out of the breeding season, regression was demonstrated, manifested with reduced weight of testes, reduced presence of germ cells in seminiferous tubules which were present only to the spermatogonia level. After the period of active sexual stimulation, testes atrophied, but never attained the size from the beginning of the breeding season (McDonald, www.scottemcdonald.com).

The weight measurements showed that the left testis was considerably heavier than the right counterpart regardless of the season. During the non-breeding season, the weight of testes sharply decreased compared to the same age during the breeding season. In birds with marked seasonal pattern of reproduction, the growth testes take about 45 days (Blanco et al., 2007). Differences in the size and weight between the left and right testis o birds are also reported by other researchers (Calhim and Birkhead, 2006; Denk and Kempenaeres, 2006; Noirault et al., 2006; Bull et al., 2007).

The dimensions of seminiferous tubules in birds at different ages corresponded to the weight of testes. The lower weight during the non-breeding season was due to the reduced seminiferous tubular size and higher relative presence of interstitial tissue in the testicular parenchyma.

The investigation of volumes of seminiferous tubules and interstitial tissue showed a superiority of seminiferous tubules over the interstitial connective tissue. The seminiferous tubules to interstitial tissue volume ratios in ganders during the breeding season corresponded to findings in birds from the Icteridae family (Lupold et al., 2009). The increased volume of seminiferous tubules is responsible for the observed seasonal hypertrophy of birds' testes (Pollock and Orosz, 2002).

Conclusion

The observed histological picture of testes during the breeding season in Landes ganders at various ages confirmed the occurrence of active spermatogenesis in them. During the non-breeding season, the presence of the different germ cell generations was reduced, with only spermatogonia being present. The established values of the different morphometric indices were logically interrelated and also reflected age- and season-related differences.

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Original scientific paper
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PATHOLOGY OF TESTES CELLS IN WHITE MICE AFTER IMPACT OF NOVOMEK

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Abstract

Antiparasitic agents based on Avermectin have main place amount modern veterinary products. Those agents have wide specter of activity and high efficiency. Studies in recent years show that Ivermectin based products have highest efficiency. One of most popular medicines in Russian Federation is Novomek. Due to the fact that Ivermectin is able to cause theratogenic and embryotoxic effects do not recommend using it in pregnant animals. However, information about negative effect of Ivermectin to male reproductive system in scientific literature is absent. This fact determines the relevance of our work. Investigation of karyopathic and cytotoxic effect of antiparasitic product Novomek to spermathogenic epithelium of laboratory mice was carried. Novomek was injected singly subcutaneously in doses of 0.2; 0.4 and 100μg/kg. Control group of mice was intacted. Changers in condition of cells were measured after 12 hours from beginning of experiment. Those pathologies as lagging of single chromosomes and groups of chromosomes and forming of chromosome bridges in anaphase and telophase were observed in experimental groups in comparison to control animals. Observed pathologies were directly connected with damages of chromosomes and may cause genetic heterogeneity of daughter cells. Also they cause disorders of process of division and cytokinesis. Under increasing of Novomek dose amount of cells with agglutination of chromosomes increase significantly. Thus, karyopathic and cytotoxic effect of Novomek single injection to spermathogenic epithelium of experimental animals is established. Therefore, that product may be used carefully to breeding animals in reproductive season.

Keywords: *Pathology, tested cells, white mice, Novomek.*

Introduction

Antiparasitic agents based on Avermectin have main place amount modern veterinary products. Those agents have wide specter of activity and high efficiency. Studies in recent years show that Ivermectin based products have highest efficiency. Due to the fact that Ivermectin is able to cause theratogenic and embryotoxic effects do not recommend using it in pregnant animals. However, information about negative effect of Ivermectin to male reproductive system in scientific literature is absent. This fact determines the relevance of our work. The aim of our investigation was research of degree of karyopatic and cytotoxic effects of Novomek to mice testis cells division.

Materials and methods

Investigations were carried out on males of white mice at 18-20g weight. Novomek was injected singly subcutaneously at doses of 0.2; 0.4 and 100μg/kg. Control group of mice was intacted. Every group consisted from 5 mice. Animals after 12 hours were decapitated. After necropsy testes were allocated and stamp-smears were prepared which were fixed by May-

Grunvald and painted by Romanovsky-Giemsa. Subsequently stamps were microscoped and a counter of devising cells was estimated. The form, size and color of nuclei were accounted. Analysis of incidence of cells with karyopatic and cytotoxic damages was investigated on detached and flattened cells with count not less than 1000 cells in every stamp. We used meiotic index (MI) is a percentage of devising cells to common number of cells. Micronuclei were identified like a round chromatins bodies wit smooth margins and size about 1/3 of nucleus which lay separately from base nucleus, did not brake light and were situated in one plane with base nucleus (Sivkova, 2011). Furthermore, dual nuclei, fragmentation and vacuolization of nuclei, earlier divergation of chromatids in prophase, pathologies of division and citotomia were identified. For the statistical processing we used software package STATISTICA 6.

Results and discussion

Data of analysis of karyopatic changes in experimental and control mice are presented in diagrams.

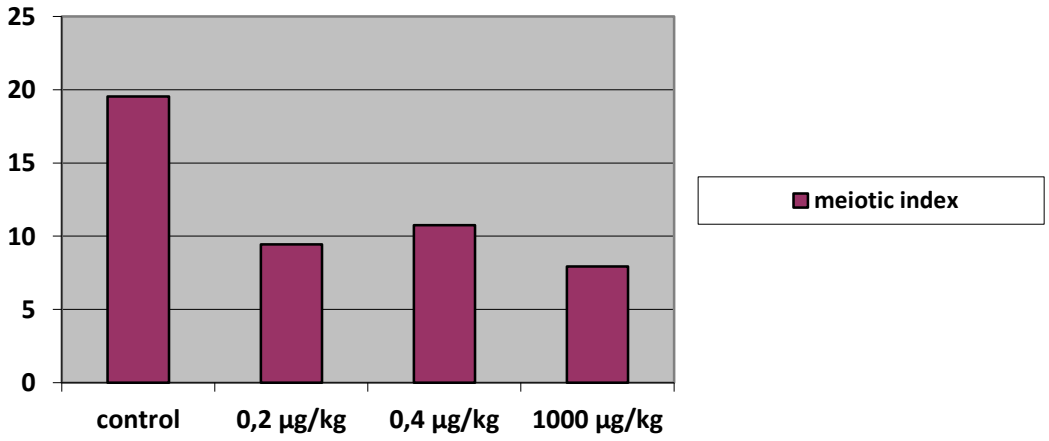

Fig 1 Changes of meiotic index (%) after 12 hours after Novomek injection in testis cells of white mice

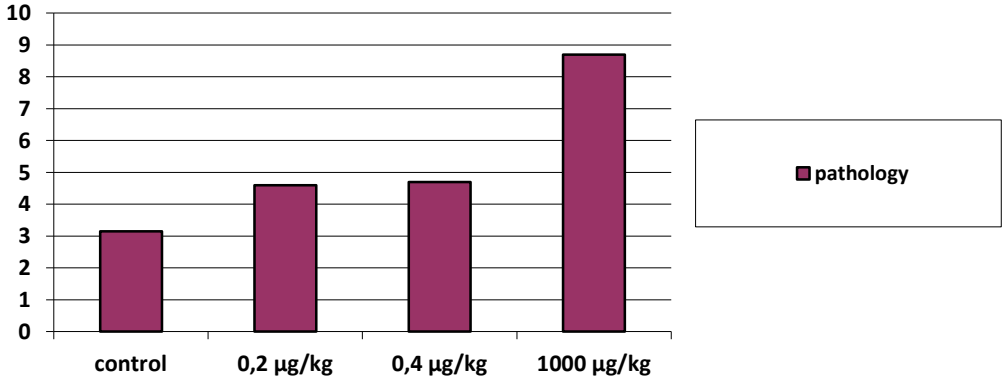

Fig. 2. Changes of pathology of meiosis (%) to amount of meiotic cells in testes of white mice at 12 hours after injection of Novomek

It is known that pathological mitoses in normal tissues in 3% of cells and less are found (Alov, 1972). Authors of this work observe high proliferative activity of testis cells (MI, %) in control group of animals (Fig.1). Thus metaphase with lagging of chromosomes and ana- and telophase with lagging of chromosomes and groups of chromosomes were in the leading place

among observed pathologies but were in limits of physiological norm. Activity of cells division in experimental groups after Novomek injection decreases more than twice, an increase of doses effects to changing of MI not significantly.

Percentage of pathologies ranged within normal limits at the all time of experiment. Thus growth of amount of pathologies to number of divising cells determines of dose (Fig.2).

When comparing the ratios of the figures we observed the predominance of metaphases and prophases over anaphases and telophases, which indicate a blocking of the process of cell division in the early stages of meiosis. In comparison with the intact group increases the number of cells with bridges in anaphase and telophase. This pathology progresses with increasing doses of Ivermectin. Abnormal findings can be directly linked with damage to chromosomes and, as a rule, lead to genotypic heterogeneity of daughter cells and breaks during the final stages of division and inhibits cytokinesis (Sivkova, 2011).

The number of cells with the agglutination of chromosomes increases significantly. This pathology is often found in tumor cells and after influence of mitotic poisons. Swelling, chromosomes lose the right shape and is bonded surfaces, form irregularly lumpy mass. Divergation of chromosomes not occures, and cell dies (Alov, 1972). During the experiment we recorded pathology such as multipolar meiosis, lagging chromosomes, groups of chromosomes at all stages of cell division. These pathologies are associated with chromosome damage and the staff of the division that breaks during meiotic cytokinesis and delays, and may cause the death of cells.

Conclusion

Analyzing the results, we concluded that the Novomek substantially inhibits the activity of dividing spermatocytes, which can lead to poor fertility of animals. Range of karyopatic effects in the experimental groups was much wider in comparison with the control group, two types of pathology (agglutination of chromosomes and ana - telophase with bridges) was in direct proportion to the increase of dosage. Obtained in the experiment, the data allow us to talk about the negative impact of Ivermektin on the reproductive system of males and the need for controlled drug pedigree animals. We recommend not use drugs based on the Ivermektin for a few days before the use of breeding males.

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Original scientific paper
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COMBINED INFLUENCE OF BACILLUS SUBTILIS AND FASCIOLA HEPATICA SOMATIC EXTRACT TO SPERMATHOGENIC EPITHELIUM OF LABORATORY AND FARM ANIMALS

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Abstract

Research of pathogenic influence of bacteria and trematoda was performed on laboratory mice and sheep. The first group of mice was fed with probiotic *Bacillus subtilis* in therapeutic doses in a period of 10 days. The second group was once intraperitoneal injected by *Fasciola hepatica* extract in dose of 100μg. The third group was fed by probiotic and injected by helminthes extract. The control group was intact. Sheep were spontaneously infected with *Fasciola hepatica* and also we used *B.subtilis* for desoldering in 10 days period. From laboratory animals testes were obtained. Smears prints of testes were colored by Romanovsky-Giemsa method. Amount of normal and pathology figures of meiosis were counted. Other part of testes were stained in 10%-formalin and used for standard histological assay. Testes of experimental sheep were obtained after emasculation and were held histological procedures.

Karyopatical and histological investigations showed that the feeding with *B. subtilis* does not induce notable changes in condition of spermathogenic epithelium. However, intraperitoneal injection of *F. hepatica* extract to laboratory mice had negative effect on cells. Joint influence of both biological agents also caused karyopatic changers. Mitotic index reduced and amount of pathologies reach the high level. Dystrophic and scleroplastic changes in experimental mice and infected sheep were occurred after feeding of probiotic. Therefore, bacterial culture using during treatment of fasciolosis is not justified.

Key words: *Bacillus subtilis, testes, division, pathomorphology.*

Introduction

Nowadays, including of different probiotics into the therapy of parasitic diseases is recommended for introduction the antagonistic bacteria to host's organism. One of those biological products is Sporovit from antagonistic active strain of *Bacillus subtilis* 12B. The high affectivity and economic benefit of those bacilli is established at infections and parasitic diseases (Gavrilieva, 2013; Neprimerova and Sivkova, 2012; Timoshok, 2012) However, information about cooperative effect of helminthes and *B.subtilis* metabolites absent in Russian and foreign literature.

Due to wide distribution of helminthiasis in farm animals, the aim of our scientific work was investigation of karyopatic effect of Sporivit on reproduction cells of animals. As fasciolosis is a one of most dangerous helminthiasis with worldwide distribution males of white mice after once introperitoneal injection of *Fasciola hepatica* extract were chosen as a laboratory model.

Materials and methods

The first group of mice was fed with probiotic *Bacillus subtilis* in therapeutic doses at the 10th day. The second group was once intraperitoneal injected by *Fasciola hepatica* extract in dose of 100μg. The third group was fed by probiotic and injected by helminthes extract. The

control group was intact. Conditions of living and feeding of experimental mice were remained at the all period of work and meet hygienic requirements.
After 48 hours from injection of extract, the animals were killed by the method of cervical dislocation. From laboratory animals testes were obtained. Smears prints of testes were colored by Romanovsky-Giemsa method. Amount of normal and pathology figures of meiosis were counted. Other part of testes were stained in 10%-formalin and used for standard histological assay.
Investigations on farm animals were carried out on the Romanov sheep breed 4 months old naturally infected with helminthiasis. After 10-days feeding of Sporovit in therapeutic doses lambs were emasculated by the open method and testes were collected for histological examination. Testes from animals stained in 10%-formalin immediately and used for standard histological assay.
The wiring of tissues and organs by the growing spirit of the fortress was carried out by hystoprocessor (Leica TP 1020) with automatic cycle within 18 hours. After ending of the wiring material was flood into the paraffin medium Hystomix using Thermo Scientific Histostar. Slices thick of 2-3μm were made from paraffin blocks using semiautomatic microtome Leica. Prepared slices were painted with hematoxylin and eosin and by Van Gison method. Painted specimens were investigated using light microscope Leica with eyepiece magnification of 10 and lens magnification of 10, 40 and 100. Most interesting objects were photographed using visual analysis system of picture by digital camera Infinity 1 and kept in the computer's memory.

Results and discussion

General condition of control and experimental animals stained good at the all period of experiment. Results of cells division activity changing are presented in diagram.

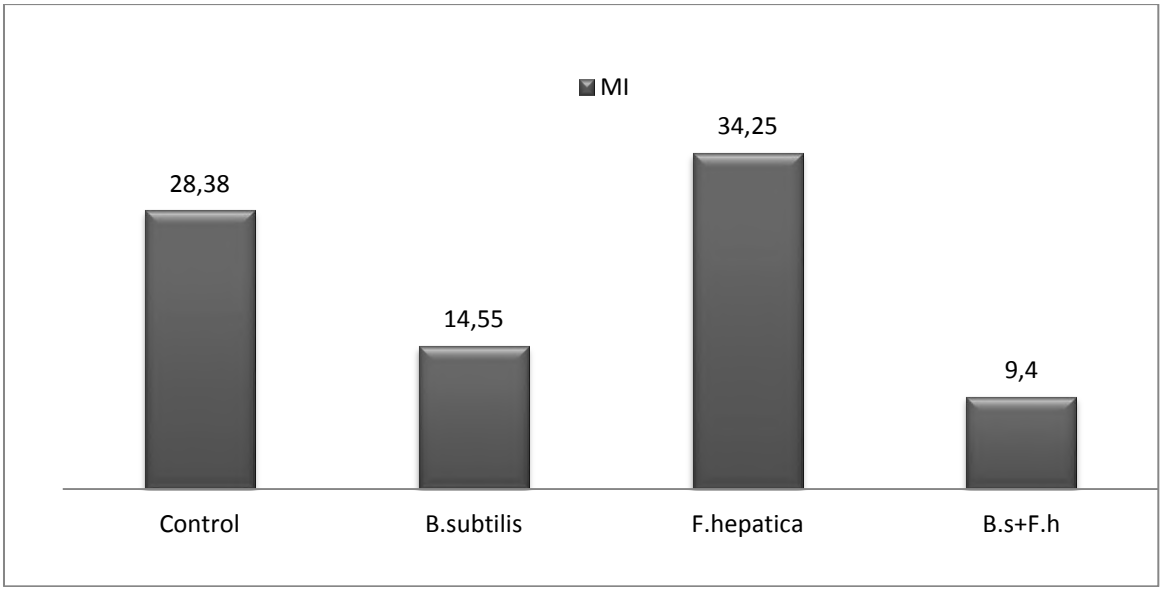

Diagram. Frequency of karyopatic consequence in mice testes after *F.hepatica* extracts injection and product of *B.subtilis* feeding

Under *F.hepatica* somatic proteins affect we observed disturbance of the process of dividing spermatogenic epithelium and growth of meiosis index (MI) after 48 hours in comparison with control data. Amount of pathology figures of division also exceed twice a control value.

Premature divergence of chromosomes in metaphase and the tripolar anaphase were detected more frequently among pathology figures of division.
Ten-day feeding with biological product of *B. subtilis* reduced the meiosis index in the testis of experimental mice, thus, level of pathology figures was approximately twice higher than the control value. The most pathological changes apparent as the backlog of individual chromosomes in metaphase and anaphase. Those anomalies occur as a result of damage of the mitotic spindle. The level of other pathological changes did not exceeded of normal physiological level.

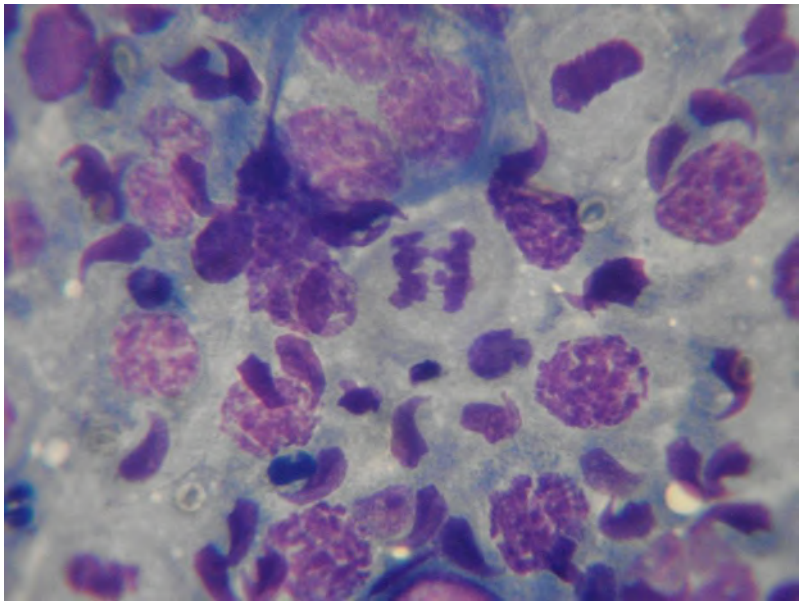

Fig 1. Testis of mouse. Anaphase with lagging of chromosomes and chromosome bridge (10x100)

Special attention should be paid to the detection of anomalies such as agglutination of chromosomes such. In this pathology so severe damage to chromosome structure occurs, that chromatin loses its ability to normal helix, but instead stick together in a shapeless mass, and further cell division is blocked. However, the number of cells with tendency to agglutinate chromosomes was small.
Based on the above observations, it follows that karyopathic effect of *B. subtilis* in relation to spermatogenic epithelium occur to a small extent. Formed due to pathogenic exposure to defective sexual products are not able to function normally and are eliminated from the body.

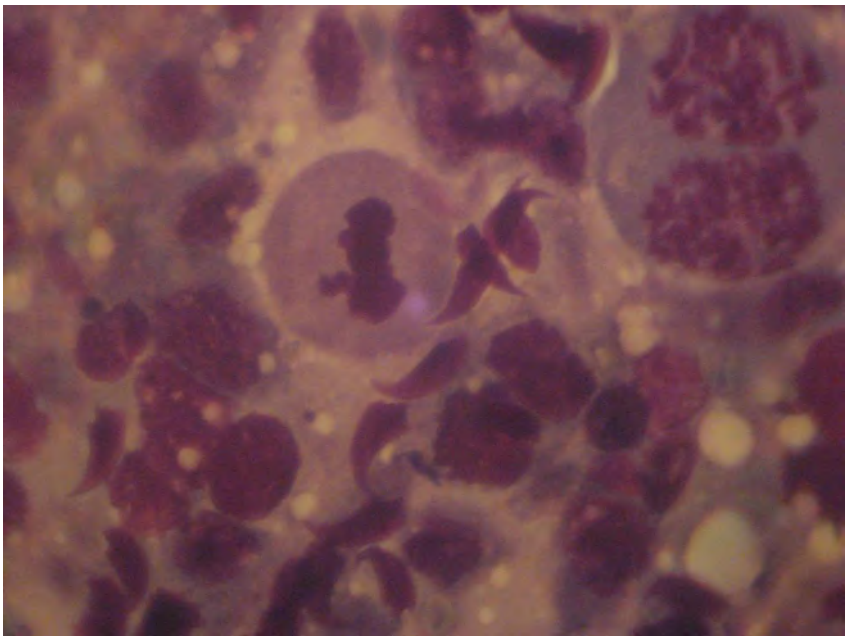

Fig 2. Testis of mouse. Premature divergence of chromosomes in metaphase (10x100)

The feeding of Sporovit to white mice in a period of 10 days before injection of the extract in 3 times reduced the meiosis index of seminiferous epithelium. The number of pathology division in the experimental group of animals was two times higher than in controls, and did not differ significantly from the results obtained from the extract of *Fasciola* without watering probiotic. The number of individual pathologies associated with abnormalities of the staff of the division, was not beyond physiological values. Therefore, changes were not specific and directional in nature. Meanwhile, the number of metaphases with premature chromosomes under the simultaneous action of *F. hepatica* and *B. subtilis* decreased by half compared to monoeffect of trematodes extract, however, was higher than in the case of the impact of the biological product Spirovit. Chromosome damage in the form of agglutination in all cases was absent.

The appearance of cells with micronucleus is significant because it is absent in previously experiments. Forming of micronucleus suggests the fact that process of cell's division was not blocked and meiosis finished with damage of nucleomorphology.

Thus, we can expect that biological active substances from probiotic strain of *B.subtilis* do not have their own high karyopathic effect on reproductive cells of laboratory animals but are not able to reduce karyopathic effect of *F.hepatica* extract. Therefore, suitability of Sporovit treatment in complex therapy of fascioliasis was not suggested.

Information about pathology of reproductive system of farm animals at fascioliasis is practically absent. Since we established after karyopatic assays a negative effect of *Fasciola* extract on division of spermatogenic epithelium the question about morphology of organs was detected.

Histological investigation showed that stromal edema of the testis of laboratory animals after once injection of *F.hepatica* extract occurred. Spermatogenesis decreases significantly and as a result a part of tubules empties. In the retained activity tubules sperm cells are fragmented and are in a state of agglutination. Founding changes indicate a significant decrease in reproductive function in experimental animals.

Pathomorphological changes were detected in testes of mice after application of *B.subtilis* culture. Normal tissue of testis was visible at the all parts of organ. The structure of spermatogenic epithelium was relatively preserved, but not in all tubules. Division of cells was expressed unevenly and in some parts was weak.

Often the tail parts of spermatozoa were in agglutination and the presence of separately located nucleated and the tail parts were detected. Some tubules were lined by a single layer of flattened cells, contained in the lumens group of sperm and single discretionary cells of seminiferous epithelium. In the stroma of the organ was detected a small group of randomly located fibroblasts.

Also we registered damages in mice testis after simultaneously effect of *F.hepatica* extract and *B.subtilis.* External layer of testis was thick and particularly delaminated due to edema. Despite the fact that normal tissue of the testis was present in all fields of view, and the layer structure of seminiferous epithelium in the tubular part was relatively preserved, often noted agglutination the tail parts and the fragmentation of the sperm cells. In the central parts of some tubules from the ruins of the sexual products were formed thin filaments. Some tubules were lined by a single layer of flattened cells, contained group of sperm and single discretionary cells of seminiferous epithelium. In the stroma were randomly located small group of fibroblasts. Therefore, the effect of biologically active substances of *B. subtilis* and somatic extract of *F. hepatica* leads to the development of dystrophic and scleral plastic processes in the reproductive organs, and adversely affects the formation of sexual products.

The study of the tissue of the testes of lambs after watering of Sporovit have caused particular interest. The outer shell is much thickened, and presented fibrous structures with large full-blooded thick-walled arterial blood vessels and thin-blooded veins. Visible areas angiomatosis are presented. The layer structure of seminiferous epithelium can be traced clearly. Distinguishable single mitoses were in the basal layer cells. Cells are in a state of degeneration, focal epidermal desquamation.

Spermatogenesis cannot be observed in all tubules, significantly weakened, sometimes marked the tail parts agglutination of spermatozoa, the presence of separately located nucleated and the tail parts in the form of a thin eosinophilic filamentary structures and eosinophilic masses of character. Stroma with marked edema, partially sclerotic, contains single thick-walled vessels of weak blood supply. In appendage – common scleral plastic changes, dilated ducts, sometimes significantly. In the ejaculatory ducts was not content. Pronounced degenerative changes of seminiferous epithelium were visible. Common peritonectomy sclerosis on the appendage with atrophic changes in the epithelium of the VAS deferens occurred.

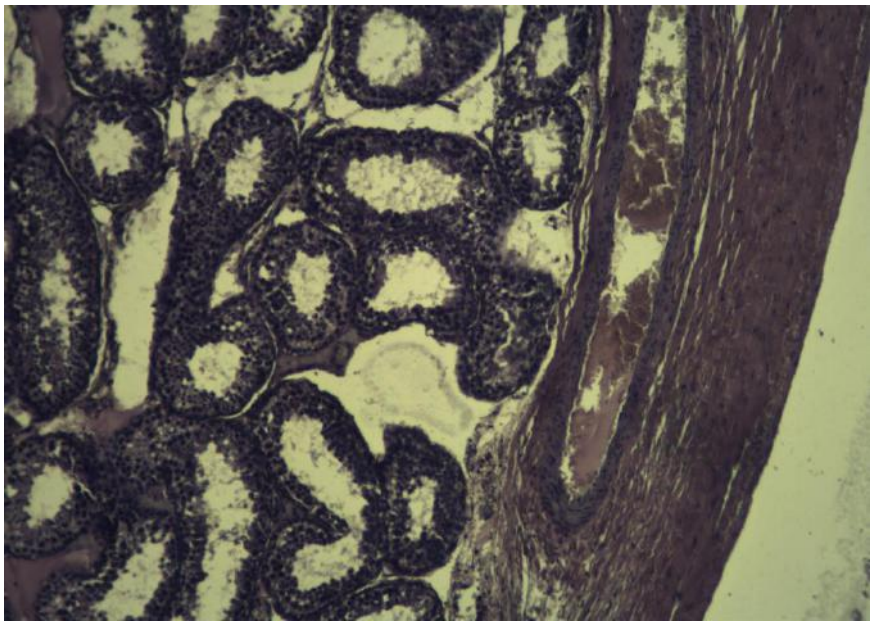

Fig 3. Testis of lamb. Dystrophy and focal desquamation of epithelium. Blood vessel with weak filling (10x10)

Conclusion

Our studies confirm a marked karyopathic effect of the *Fasciola* extract and culture of *B. subtilis* 12B on the seminiferous epithelium of laboratory and farm animals, which manifests itself in increasing the number of abnormalities of cell division. On the background of experimental and spontaneous effects of the products of metabolism of helminths after oral application of probiotic preparation in the testes occur degenerative and scleroplastic processes occur, which negatively affects the reproductive function of animals.

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Original scientific paper
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COMPARATIVE EVALUATION OF REPRODUCTIVE QUALITIES OF LARGE WHITE BREED OF SOWS ON OFFSPRING QUALITIES

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Abstract

In today's swine breeding, we should highlight the major economic indicators, on which the efforts of all professionals involved in the pork production technologies are aimed. This is the organization level of intense reproduction of pigs, including expanded reproduction, making maximum use of the reproductive capacity of the breeding stock, increasing productivity, increasing the livability of youngsters. In breeding Large White breed of swine, which is the main maternal breed in most systems of crossbreeding and hybridization, great attention is paid to the selection of sows that possess the highest reproductive qualities. Their offspring is bred for rearing stock and for creating and improving lines and family. The aim of our study was to conduct a comparative assessment of the reproductive performance of Large White breed of sows on the qualities of their offspring, the study of heritability and the correlation between reproductive traits in hybrid breeding in conditions of the Selection Center of the OJSC "Permskii Swine Complex" in Krasnokamsk District of Perm krai. On the basis of the carried out research we established that the genetic similarities between daughters and mothers by straight-line correlation showed that the highest rate of heritability was recorded on fetal macrosomia, i.e. the variability of this trait is more influenced by the difference of genotypes. We noticed that other productivity traits (prolificacy, nest mass at birth, lactescence, the number of heads to weaning, nests mass before weaning) had low heritability rates, i.e. the variability of these traits is influenced by housing conditions.

Keywords: *swine production, Large White breed, sows*

Introduction

In today's swine breeding, we should highlight the major economic indicators, on which the efforts of all professionals involved in the pork production technologies are aimed. This is the organization level of intense reproduction of pigs, including expanded reproduction, making maximum use of the reproductive capacity of the breeding stock, increasing productivity, increasing the livability of youngsters (Khlopitskii, 2009; Filatov, 2002; Vangen, 1986);

The efficiency of reproduction is largely determined by the cost of herd breeding and management. It is therefore important to select high-yielding primipara sows, which in the future will preserve high breeding qualities [5, 10]. Effectiveness of screening and selection process depends heavily on the accuracy and objectivity of evaluation indicators of productive qualities heritability. Since heritability is not only a property of characteristics, but also of populations, it is necessary to determine its level in each specific condition for each individual groups or lines (Garai, 2007, 2004; Dudka, 2002; Buchova, 2010)

Large White breed of swine is widely recognized as one of the best, most specialized selected on a number of commercially useful and technological characteristics. In this regard, it is widely spread in our country, including in the area of the Urals.

In selection of Large White breed of swine, which is the main maternal breed in most systems of crossbreeding and hybridization, great attention is paid to the selection of sows that possess the highest reproductive qualities. Their offspring is bred for rearing stock and for creating and improving lines and family. Among the main factors that affect the efficiency of

selection, inheritance is also of great significance to those characteristics by which to filter. From practice, it is known that selection of animals only by phenotypes does not contribute to the qualitative improvement of herd for a long time, and not always from highly productive parents the same valuable offspring are obtained. Selection by related or own animal productivity is effective only for those characteristics that have high inheritance. Quantitative traits are characterized by low level of inheritance, and therefore require more advanced selection methods – evaluation of parental genotypes on quality of offspring, individual selection, determining the best combinations (Rybalko and Birta, 2009; Shtakelberg, 2005; Peškovičová, 1998).

Selecting parent types and lines is carried out primarily by reproductive qualities, multifetation, safety, ability of sows to display their mothering qualities well. Purpose of maternal lines – compilation of breeding stock at all levels of hybridization programmes (Gaisbertus, 2008; Suslina, 2010).

The aim of this work is to assess reproductive qualities of sows of Large White breed.

To achieve this goal, the following tasks were solved:

To carry out a comparative analysis of reproductive qualities of mothers and daughters in pure-bred Large White breed of swine.

To study the characteristics and correlation of reproductive traits in mothers and daughters.

Materials and methods

Investigations were conducted in conditions of the Selection Center of the OJSC "Permskii Swine Complex" in Krasnokamsk District of Perm krai on pure-bred sows of Large White breed (254 heads). The data of pedigree animal cards served as material for research. Assessment of reproductive qualities were conducted by generally accepted methods and indicators. The study examined productivity of mothers and daughters, as well as the characteristics and correlation of such traits as multifetation, macrofetation, lactescence, number of piglets and sows, piglet weight at weaning. At the time of the research animals were in identical conditions of housing and feeding. Generated databases were processed statistically in Excel.

Results and discussion

The average duration of use of all sows that were used in the experiment was 3.64 ± 0.06 of farrowing. For the full sample ($n = 254$) obtained data on the productivity of mothers and daughters of Large White breed is presented in table 1.

In the evaluation of their own productivity while achieving 100 kg of live weight, precocity in daughters made up 192.8 days, 3.9% higher compared to the precocity of mothers. Fat thickness over 6-7 breast vertebra in daughters was 2.3 cm, that is 0.06 cm less than in mothers. Body length of mothers was at 1.0 cm more than body length of daughters. Therewith it was established, that daughters surpass mothers on macrofetation by 0.07 kg, lactescence by 2.8 kg, the number of piglets at weaning by 0.5 heads, when nests mass at weaning aged 2 months by 5.8 kg.

Table 1. Productivity of mothers and daughters of Large White breed

Indicator	Mothers	Daughters
Assessment per 100 kg		
Precocity, days	200.60 ± 0.32	192.80 ± 0.33
Fat thickness over 6-7 breast vertebra, cm	2.36 ± 0.07	2.30 ± 0.07
Body length, cm	123.20 ± 0.07	122.20 ± 0.07
Data on farrowing		
Multifetation, heads	13.20 ± 0.04	12.10 ± 0.06
Nest mass at birth, kg	18.60 ± 0.05	17.80 ± 0.08
Macrofetation, kg	1.42 ± 0.03	1.49 ± 0.04
Data on lactescence		
Quantity, heads	9.90 ± 0.02	10.60 ± 0.04
Lactescence, kg	65.60 ± 0.19	68.40 ± 0.25
Mass of 1 animal, kg	6.58 ± 0.01	6.49 ± 0.20
Data on weaning		
Quantity, heads	10.00 ± 0.20	10.50 ± 0.03
Mass of nest aged 2 months, kg	224.10 ± 0.58	229.90 ± 0.79
Mass of 1 animal, kg	22.30 ± 0.04	21.90 ± 0.06

At the same time, mothers maintain supremacy for multifetation by 1.1 head per farrowing, nest mass at birth by 0.8 kg and for 1 animal weight at weaning in the age of 2 months by 0.4 kg. Thus, daughters having a lower multifetation, but better lactescence, preserve to weaning more by 5.0% piglets.

When calculating the heritability ratio based on the straight-line correlation method was used the formula $h^2 = 2rd/m$. Heritability ratio is equal to double coefficient of phenotypic correlation between characteristics of mothers and their daughters. This formula applies to the cases where differences in the diversity of traits in parents and offspring are insignificant. Heritability ratio is widely used in the practice of selection work. It is used to predict the efficiency of selection. Heritability ratios of reproductive traits in pigs are presented in table 2.

Table 2. Heritability ratios of reproductive traits in sows

Indicator	Heritability ratio
Multifetation	0.22
Nest mass at birth	0.30
Macrofetation	0.58
Lactescence	0.28
Quantity of animals before weaning	0.06
Nest mass before weaning	0.26

In determining the genetic similarities between daughters and mothers by the method of straight-line correlation it was found that the greatest coefficient of heritability was recorded on macrofetation, i.e. the variability of this trait is more influenced by the difference of genotypes. In other characteristics of productivity (multifetation, nest mass at birth, lactescence, number of animals to weaning, nest mass to weaning) a low value of heritability coefficients was observed, i.e. the variability of these traits is affected by housing conditions.

Study of correlation relationship showed that among individual reproductive traits there is a positive or negative relationship (table 3).

Table 3. Reproductive traits correlation coefficients

Indicator	Mothers	Daughters
Multifetation × macrofetation	-0.46	-0.44
Multifetation × lactescence	-0.14	-0.05
Multifetation × quantity of piglets at weaning	0.03	0.02
Multifetation × nest mass at weaning	-0.11	-0.04
Macrofetation × lactescence	0.41	0.37
Macrofetation × quantity of piglets at weaning	0.13	0.16
Macrofetation × nest mass at weaning	0.36	0.37
Lactescence × quantity of piglets at weaning	0.61	0.65
Lactescence × nest mass at weaning	0.82	0.84
Nest mass at weaning × quantity of piglets at weaning	0.69	0.70

The most important phenotypic correlation coefficients were observed in sows-mothers between such traits as macrofetation and lactescence (r = + 0.41), macrofetation and mass of nests at weaning (r = + 0.36), lactescence, the number of piglets at weaning and mass of nests at weaning (r = +0.61, + 0.82), weight of nests and the number of piglets at weaning (r = + 0.69). Positive and reliable correlations for sows-daughters were established between their macrofetation and lactescence (r = + 0.37); macrofetation and mass of nests at weaning (r = 0.37), lactescence, the number of piglets at weaning and mass of nests at weaning (r = + 0.65, + 0.84), the presence of live pigs to weaning and mass of nests at weaning (r = + 0.70).

Conclusions

Based on the research the following conclusions are drawn:

The lack of statistically reliable indicators of heritability suggests that the variability is phenotypic traits considered depends mainly on the influence of environmental conditions and weakly determined genetically.

Analysis of the relationship of reproductive traits in sows suggests that in populations of Large White breed of swine, reliable correlation coefficients were obtained between macrofetation, lactescence and mass of nest at weaning; lactescence and the number of piglets at weaning and mass of nest at weaning; between the mass and the number of nests piglets at weaning, which gives the opportunity to engage in effective selection of one of these traits.

Use in further selection work received genetic herd parameters of Large White breed of swine will increase the effect of selection.

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CARBOHYDRATES FRACTIONS BY CNCPS OF PEA-OAT MIXTURE

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Abstract

Inadequate content and structure of carbohydrates in livestock diet can cause metabolic disorders that, in turn, may lead to long-term effects on health and productivity. The appropriate structure of carbohydrates in the diet is very important for digestion in the rumen and to provide an adequate amount of energy for microbial protein yield and maintenance of a stable level of fermentation. This research was conducted to determine the effects of seed rates in the mixture forage pea + oat. Also, aim was to define cutting stages on changes in the structure and ratios of fractions of carbohydrate biomass mixture. We used two different rates of pea and oat (2/4 Pea: 2/4 Oat- mixture R_1, 3/4 Pea : 1/4 Oat- mixture R_2) and three different cutting stages of early flowering of field pea stage- phase P_1, pods forming stage -phase P_2, early seed forming stage-phase P_3). The results show that the ratio of crops did not significantly affect the amount of total carbohydrates (CHO) and lignin (ADL), and the mixture with a higher proportion of pea had significantly higher content of non-structural carbohydrates (NFC). The minimum lignin content was determined in flowering pea, while significantly higher content was recorded in the second and third stage of the pea development. Analysis by CNCPS model showed that both factors had a significant impact on the change in the ratios of certain fractions of carbohydrates. Fraction CB makes the biggest part of all fractions and with CB_2 and CB_3 together makes up over 50% of the total fraction. Fraction CC, which is related to lignin and unavailable to the animal significantly increased with phase-change cutting (164.0 g kg^{-1} CHO in phase P_1 to 181.9gkg^{-1} CHO in phase P_3).
Keywords: CNCPS, pea, oat, fractions, biomass.

Introduction

With the development of the scientific field of animal nutrition, many nutritional models have been created and perfected. Most nutritional models are based on the needs of different animal species and categories for a specific concentration of certain nutrients in the diet. They, as a base for nutritionally balanced meals, take the amount of energy the animal enters through the meal which is needed for the animal to perform its vital functions and production. Considering that the main sources of energy in feed for ruminants are carbohydrates, it can be understood how important the representation of certain forms of these nutrients in the diet is.

Carbohydrates (CHO) are a major component of net energy needed for maintenance of the basic life functions and milk production in ruminants. In addition, they are the main source of energy for the growth of microorganisms in the rumen and the production of microbial protein. Carbohydrates make up over 65% of dry matter and the largest part of nutrients in dairy cow meals (Varga et al., 1998). The amount and the representation of all forms of carbohydrates in the diet affect the composition of milk, as they are a precursors for lactose, fats and proteins.

In the plant structure, carbohydrates are represented in two basic forms as structural (FC) and nonstructural carbohydrates (NSC). Inadequate content and ratio of these substances in the diet can cause serious metabolic disorders (acidosis, abomasum dislocation, ketosis), which can cause long-term effects (Marković, 2015). The differences in the amount and chemical

characteristics of FC and NSC in the diet can affect the performance of the animals. High levels of fiber in the diet reduce digestibility and limit the capacity of rumen for other nutrients (Mertens, 1997). The lower intake of digestible NCS reduces milk production. On the other hand, with low levels of fiber in the diet, there is a negative effect on the fermentation in the rumen (Lanzas, 2007).

The most important factor affecting the nutritional value and the representation of certain nutrients in biomass is the stage of plant development at the moment of cutting for consumption or conservation. As the plant becomes more mature, the content of protein, energy, calcium and phosphorus is reduced and ratio of fiber is increased. This is accompanied with an increase in the percentage of lignin, which is indigestible and unavailable to the animal, resulting in decrease of the energy value of nutrients (Zeremski et al., 1989, Grubić et al., 1996).

The decline in the nutritional value of plants with their maturity occurs due to reduced digestibility of leaf and due to the increase of the ratio of stems as less digestible parts with the higher proportion of fiber, especially lignin, and due to the flattening and rotting. Along with the decline in nutritional value, the energy value of the feed reduces (Grubić and Adamovic, 2003). In addition, the nutritional value of forage is influenced by other factors as well. So, there may be significant differences in the chemical composition of certain plant species, but, also, within cultivars of the same species. Consequently, the prevalence of specific plant species in the mixtures can significantly influence the share of individual carbohydrate fraction in biomass

The amount of individual fractions of CHO, FC and NSC in plant material is of great importance for the passage of these nutrients through the digestive tract. Their relative share in the structure of carbohydrates determines the rate of degradation and the possibility of using these nutrients by animal itself and by microorganisms of the rumen.

The aim of this study is to evaluate the changes in the amount of CHO and representation of individual fractions in biomass of mixtures of annual legume and cereal, depending on the stage of exploitation and the ratio between cereal-legume in the mixture. The results will give an insight into the possibility of combining this kind of feed with other bulk meals when balancing the diet of different species and categories of ruminants.

Materials and method

Binary mixtures of field pea and oats are sown on the experimental plot of the Institute for forage crops Kruševac, Serbia. Two factorial experiment was established in autumn in 2012, on October the 20th by randomized block design with three replications per treatment. The first factor (A), is ratio of germinated seed in mixtures. The ratios were 120 pea seeds and 120 oat grains in the first mixture (A1 – 2/4:2/4) and 180 pea grains and 60 oat grain in second (A2 – 3/4:1/4). The second factor is a phase of cutting of biomass with three sub-stages: B1 – a cutting of biomass at the start of flowering pea (10% of flowering), B2 – a cutting of biomass at forming the first pods on 2/3 plants of pea, and B3 - cutting of biomass at forming green seeds in 2/3 pods. Both mixtures were sown on plots of 20 m^2 with three replications for each stage of cutting. With each repetition has been taken by one kilogram of green (fresh) biomass for chemical analysis.

In DM of mixture., the amount of total carbohydrates (CHO), non-fibrous carbohydrates (NFC), monosaccharides and disaccharides (TESC), acid detergent fiber (ADF), neutral detergent fiber (NDF) and the amount of lignin (ADL) were determined.

Crude carbohydrate (CHO) cell fractionation was performed according to the Net Carbohydrate and Protein System. The characterization of the CHO fractions was applied in this system. Simple sugars which ferment very quickly were designated as fraction of CA carbohydrates. In addition to simple sugars, this fraction contains organic acids, which are

structurally most similar to carbohydrates. CB fraction of CHO was the largest faction in nutrients. It includes a large variety of different compounds with variable degradation speeds, solubility and degradability. Considering the degradation speed it was divided into three sub-fractions: CB_1, CB_2 and CB_3.

Sub-fraction CB_1 was represented by starch which was largely broken down in the rumen and is accessible to animals. Sub-fraction CB_2 was soluble fiber, consisting of β-glucans and pectin substances that are defined as dietary fiber, because they can not be digested by the mammalian enzymes. Slow-degradable sub-fraction CB_3 was available cell wall with a very low rate of degradation. Fraction CC was the inaccessible part of the cell wall of which the largest part is lignin.

The analysis of variance was conducted using the results of chemical analysis of silage quality. The statistically significant differences for all tested parameters between treatments were tested by LSD test (StatSoft, 2006).

Results and discussion

By the chemical analysis of the mixtures, the content of structural and non-structural carbohydrates in dry matter of DM biomass was determined, as well as trends in change in the representation of these fractions by cutting stages (Table 1).

The ratio of legumes and cereals did not significantly affect the amount of total carbohydrates, while the stage change and the maturing of plants influenced the significant increase of their quantity. In both mixtures the amount of CHO was over 700 gkg^{-1} DM, which is in line with the results of Das et al. (2015).

Table 1. Structural and non-structural carbohydrates in biomass of pea-oat mixtures $g\ kg^{-1}$ DM

Treatments		CHO	TESC	STARCH	NFC	NDF	ADF	ADL
A_1	B_1	678.3c	199.8a	38.75e	333.1d	550.11a	418.9a	70.6bc
	B_2	705.6b	176.9b	67.35a	317.9e	564.2a	415.1a	69.8bc
	B_3	736.6a	98.4f	43.40d	345.9c	548.8ab	393.3b	75.9ab
A_2	B_1	675.1c	142.5d	45.10c	447.7a	434.3d	383.2b	66.1c
	B_2	711.3b	131.5e	43.61d	367.8b	520.5c	417.3b	82.2a
	B_3	722.5ab	151.6c	58.10b	370.7b	530.5bc	361.8c	73.0bc
$\overline{X}$ A_1		706.8a	158.4a	49.83a	332.3b	554.3a	409.1a	72.1a
$\overline{X}$ A_2		702.9a	141.9b	48.93a	395.4a	495.1b	387.4b	73.7a
$\overline{X}$ B_1		676.7c	171.2a	41.92c	390.4a	492.2b	401.1b	68.3b
$\overline{X}$ B_2		708.5b	154.2b	55.49a	342.9c	542.3a	416.2a	76.0a
$\overline{X}$ B_3		729.6a	125.0c	50.75b	358.3b	539.6a	377.6c	74.5a

The total amount of CHO in the mixture biomass, NFC comprises about 45-55%. The mixture A_1 contained significantly more soluble, simple sugars (monosaccharides and disaccharides - TESC), NDF and ADF compared to the mixture A_2. The assumption is that the higher the proportion of oats in the mixture affected the greater amount of these fractions of carbohydrates in the mixture A_1. Starch and TESC in the structure of the NFC comprises about 50%. Increasing the share of pea in the mixture reduces the proportion of TESC in NFC. This is a very important considering the suitability of biomass of the mixtures for silage. These results confirm the allegations of (Đordjević and Dinić, (2007), Dinić et al., (2008) that the mixtures of peas with cereals are significantly easier to ensilage than biomass of pure pea crop because of the greater amount of readily soluble carbohydrates.

Quantity of TESC significantly decreased with the change of the utilization stage. The change of starch amount in the biomass mixture shows sinusoidal trend depending on the stage of exploitation. With the change of stage from B_1 to B_2, there was a tendency of significant increase of starch amount from 41.92 to 55.49 gkg^{-1} DM, while in stage B_3 there was decrease of the quantity of starch to 50.75 gkg^{-1} DM. The values obtained for the amount of starch in both mixtures are significantly less than those reported by Kocer and Albayrak (2012) for oat biomass. These results indicate that the pea significantly reduces the amount of starch in the biomass of the mixture compared to oat in monoculture.

The amount of NDF in bulk feed is the best single indicator of the consumption potential for ruminants. It is a reliable criterion in formulating a ration for high-production dairy cows (Marković, 2015). In the observed treatments, amount of NDF and ADF was statistically significantly higher in the mixture A^1 (554.3 and 409.1 gkg^{-1} DM) in relation to the mixture A_2 (495.1 and 387.4 gkg^{-1} DM). This is due to greater representation of oats in the mixture A_1. Oats biomass had significantly higher values of NDF and ADF in relation to pea biomass (Kocer and Albayrak, 2012, Omokanye, 2014).

Average values for NDF and ADF in the observed mixtures of pea and oats were in accordance with the results of Aasen et al. (2004). With the ripening of plants, there was a significant increase in NDF in stages B_2 and B_3 compared to the stage B_1, which is in accordance with the results of Aasen et al. (2004). The amount of ADF in the mixture biomass is increased to the stage of pod formation. At the stage of seed filling in pea, declining trend for the amount of the ADF was determined.

There were no significant differences in the level of ADL in the mixtures. This indicates that the ratio of peas and oats did not have a substantial effect on the amount of ADL in the mixture biomass. At the stage of pod formation and seed filling, there was a significant increase in ADL until the stage of flowering in pea. The obtained values for ADL both in both mixtures of peas and oats (over 70 gkg^{-1} DM) and for all three stages of utilization are significantly higher than the values stated in the NRC. Also, for similar mixtures of vetch and oats, Lithourgidis et al. (2006) showed significantly lower values for ADL (55 gkg^{-1} DM).

Table 2. Carbohydrate fraction of biomass mixture of pea-oat by CNCPS

Tretments		CA	CB_1	CB_2	CB_3	CC
A_1	B_1	294.6a	57.2d	126.5cd	352.3b	169.4c
	B_2	253.4c	95.5a	115.7d	366.3a	169.2c
	B_3	275.7b	58.9d	132.0c	351.2b	182.1b
A_2	B_1	239.8d	66.8c	246.4a	277.7e	169.1c
	B_2	188.9f	61.2d	252.9a	299.8d	197.3a
	B_3	208.7e	81.8b	187.8b	336.2c	185.4ab
$\overline{X}$ A_1		274.6a	70.5a	124.7b	356.6a	173.5b
$\overline{X}$ A_2		212.5b	69.9a	229.0a	304.6b	183.9a
$\overline{X}$ B_1		267.2a	62.0c	186.4a	315.0c	169.3b
$\overline{X}$ B_2		221.2c	78.3a	184.3a	333.2b	183.0a
$\overline{X}$ B_3		242.2b	70.4b	159.9b	344.6a	182.8a

In this model, CHO are divided into five fractions by the speed and degree of degradation in the digestive tract. Generally speaking, mixtures of peas and oats have proven to be a better source of fermentable sugars (CA fraction) compared to the results of Das et al., (2015) in their research of oat monoculture, but are in accordance with the results that same authors obtained for cowpea. These results are consistent with the results of Kamble et al. (2011) and

Gupta et al. (2011), who stated that legumes have a significantly higher CA compared to the plants of the grass family.

In contrast to these results, the same conclusion can not be drawn when comparing mixtures of legumes and cereals. Significantly higher amounts of CA (274.6 gkg^{-1} CHO) and CB_3 (356.6 gkg^{-1} CHO) fractions were found in the mixture A_1 compared to CA (212.5 gkg^{-1} CHO) and CB_3 (304.6 gkg^{-1} CHO) for the mixture A_2. In contrast, a larger proportion of peas in mixture A_2 resulted in the significantly larger amount of CB_2 and CC fractions. Das et al. (2015) reported significantly higher levels of CB_2 in oats monoculture (561 gkg^{-1} CHO) in relation to our results for the mixtures A1 (124.7 gkg^{-1} CHO) and A_2 (229.0 gkg^{-1} CHO). It can be assumed that competition between peas and corn in the mixture led to changes in the representation of certain factions in the structure of CHO. The ratio of peas and oats in the mixture had no significant impact on the amount of CB_1 fraction consisting of starch.

Change of the maturation stage led to changes in the amount of all the monitored fractions. Fraction CA and CB_1 have opposite trends with the change of time of mowing. The amount of CA fractions decreases from flowering stage - B_1 (267.2 g kg^{-1}CHO) to the stage of pod formation - B_2 (221.2 g kg^{-1}CHO) and then again there is an increase in seed filling stage - B_3 (242.2 g kg^{-1} CHO). This fact is important from the point of ensiling because lactic acid bacteria used easy-soluble sugars (CA fraction) in the fermentation process and the production of lactic acid. In contrast to CA fraction, the amount of CB fraction, consisting of starch, is the highest in the phase B_2 (78.3 g kg^{-1} CHO), while the phases of B_1 and B_3 values were statistically significantly lower. Generally speaking, CB_2 is the least represented fraction in CHO with a share of less than 8%. Fraction CB_2 had the significantly lowest value in stage B_3. The most abundant fraction of CHO in mixtures of peas and oats was CB_3 with a share of 30-35% CHO. With ripening stage change?, CB_3 fraction was significantly increased by 315.0 g kg^{-1}CHO in stage B_1 to 344.6 g kg^{-1}CHO in stage B_3. The fraction with the highest lignin content increased significantly with the beginning of pod formation in comparison to the stage of flowering which is in line with the increasing amounts of lignin in biomass mixture. Between stages B_2 and B_3 was no statistically significant difference in the amount of CC.

Conclusions

Carbohydrates are the main source of energy for livestock to perform basic life functions, milk production, the growth of microorganisms in the rumen and production of microbial protein. In this experiment, the results indicate that the stage of maturity (factor B) has a more significant impact than the ratio of peas and oats in the mixture (factor A) on the change of certain fractions of CHO, the ratio between structural and nonstructural carbohydrates. The ratio of peas and oats in the mixture did not significantly affect the amount of CHO, starch and ADL. Higher representation of NDF and ADF in mixture A_1 is due to higher ratio of oats in the biomass of mixture. Larger amounts of CA, CB_1 and CB_3 were detected in the mixture A_1, while CB_2 and CC were more abundant in mixtures with greater ratio of peas.Most abundant fraction in mixtures (30-35% of CHO) was the slow-degradable fraction CB_3, which is available cell wall with a very low rate of degradation. The amount of unavailable carbohydrates, fraction CC, was significantly higher after the formation of pods and grain filling to the stage of flowering. Stage of plant development had a significant impact on all parameters.

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RURAL DEVELOPMENT AND AGRO-ECONOMY

Original scientific paper
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AGRICULTURAL EXTENSION AND ADVISORY SERVICES IN ALGERIA

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Abstract

Algeria has a real potential for achieving food security. Agriculture sector accounts for 11% of GDP. However, despite reform policies and public investments, agricultural production fell by 30% over the last thirty years. Agricultural holdings are characterized by small sizes, low productivity and serious technical deficiencies. To address these problems, the state implemented new policies (*e.g.* Renewal of Agricultural and Rural Economy program, Agriculture Act, Law on Agricultural Orientation). The main lines of the new policy are agricultural revival, rural areas revitalisation and capacity building. The new legal framework gives a great importance to research and extension. The paper provides an overview on public agricultural extension and advisory services (EAS) in Algeria. It is based on a cross-checking of secondary information with primary data collected by a semi-structured interview with the director of the Directorate of Training, Research and Extension (DFRV) at the Ministry of Agriculture and a focus group discussion with the staff of the National Institute for Agricultural Extension (INVA) carried out in March-May 2015. The main extension program, Programme for Strengthening Human Capacities and Technical Assistance (PRCHAT), was launched by INVA in 2009. It aims at expanding the intervention capacity of public support and development structures, strengthening coordination mechanisms and decentralizing services for agriculture and rural development. Apart from advice provided by public extension agents, farmers rely also on other advisory services providers. Most of farm managers are illiterate which complicate extension work. EAS play an important role in agricultural and rural development. Therefore, political and institutional environment should be improved, coordination between researchers and extension agents strengthened and involvement of farmers fostered.

Keywords: *Agricultural extension, Rural development, Policy, Algeria.*

Introduction

Algeria has a high agricultural potential. Agriculture, with 11% of GDP, plays a very important role in the Algerian economy (Maragnani *et al*., 2012; Si Youcef, 2013). However, the low agriculture productivity makes food import necessary, only 25% of food needs are met by domestic production (Belabas, 2010). In general, crop productivity is weak compared with neighbouring Morocco, whereas the yield is at least double overseas (Bedrani, 2002). Several factors have hampered agriculture development in Algeria, including land tenure constraints, lack of investment, insufficient access to input, reduced water availability, low levels of education and agricultural training, lack of effective extension services support and marketing channel constraints (Laoubi and Yamao, 2012).

Despite policy reforms and public investments the agricultural sector, previously dominant in the Algerian economy, witnessed a 30% production fall over the last thirty years (Si Youcef, 2013). To deal with this problem, the state implemented new policies; Renewal of

Agricultural and Rural Economy program (PREAR, *Programme de Renouveau Agricole et Rural*) and new legislation as the Agriculture Act (law No. 08-16 of August 3, 2008) (MADR, 2008) and the Law on Agricultural Orientation (adopted in 2008). The main lines of this new political framework are:
- Agricultural Revival: intensification and modernization of the agriculture sector;
- Rural Renewal: watershed protection and integrated development of mountain areas, development of the Southern Highlands, control of erosion and desertification;
- Capacity Building and Technical Assistance: modernization of administration; fostering of training, extension and research; strengthening of agricultural control and protection services.

The new policy framework gives a great importance to research and extension. The Ministry of Agriculture considers agricultural extension as a cornerstone to improve farmers' technical skills.
The paper aims at providing an overview on public agricultural extension and advisory services (EAS) in Algeria.

Material and methods

The manuscript is based on a cross-checking and validation of secondary information, from different sources (*e.g.* IFPRI, GFRAS, MARD), with primary data collected by a semi-structured interview (SSI) with the director of the Directorate of Training, Research and Extension (DFRV) at the Ministry of Agriculture, Mr. Louardi Guezlane, and a focus group discussion (FGD) with the staff of the National Institute for Agricultural Extension (INVA) carried out in March-May 2015. Other semi-structured interviews were carried out with trainers of different agricultural training institutes (*ITMAS* of Medea, Ain Temouchent and Ain Taya), heads of extension services of several agricultural directorates (e.g. Chlef, Ain Defla, Tissemsilt, Algiers) and heads of extension departments of different technical institutes (National Institute of Plant Protection - INPV, National Centre of Control and seeds and seedlings Certification - CNCC, Technical Institute of Field Crops (cereals/forages) - ITGC; National Institute of Soil, Irrigation and Drainage - INSID, Technical Institute of Industrial Crops and Gardening - ITCMI). The following issues were addressed in SSIs and FGD: policy frameworks and governance, main cooperation projects, financing, human resources, gender, advisory methods and media, and monitoring and evaluation. Secondary data were collected also from quarterly, semi-annual and annual reports and daily or weekly briefings issued by different institutions mentioned above.

Results and discussion

According to Benfrid (1997), agricultural extension in Algeria is often reduced to the decomposition of the technical package, a set of topics to raise the awareness of the farmer. Farmers are considered as passive receptors. The Worldwide Extension Study (WWES) showed that Algeria has a total of 834 public extension staff of which 722 are field-level extension staff. About 82% of the field-level extension workers are male, with 65% of them holding a 2-3 year agricultural diploma. Most of the women extension workers are field-level (130 women staff) with 46% of them having a MSc/agricultural engineer degree (IFPRI, 2012).
The Ministry of Agriculture delegates extension to the Directorate of Agricultural Services. Within the institutional framework for extension the primary responsibility for program planning falls at the local and national levels and the primary linkages are agricultural schools and input supply firms. Farmer organizations have a role in helping set extension priorities and promoting farmer-to-farmer extension activities. Small-scale subsistence farmers and large commercial farmers are the clientele most targeted by extension activities

(IFPRI, 2012).
The Global Forum for Rural Advisory Services (GFRAS) identifies 13 public extension providers in Algeria (GFRAS, 2013). The main institutes and research centres that have a stake in agricultural extension in Algeria are (Korichi, 2010): National Institute of Agronomical Research of Algeria (INRAA); ITGC; ITCMI; CNCC; INPV; Technical Institute of Livestock (ITELV); National Institute of Orchards and Vineyards (ITAFV); INSID; National Institute of Forest Research (INRF); National Centre of Artificial Insemination and Genetic Breeding (CNIAAG). The High Commissariat for the Steppe Development (HCDS) and Commissariat for Agricultural Development of Saharan Regions (CDARS) are active in marginal and less favoured areas (Korichi, 2010). However, a crucial role in the agricultural extension system is played by INVA (Box 1).

Box 1. Mission of the National Institute of Agricultural Extension (INVA).

The main missions of INVA are:
1. Studies and investigations (systems, approaches and extension methodology; communication channels, means and techniques).
2. Technical support and activities in rural areas by establishment of coordination and facilitation mechanisms; developing campaigns and programs of national interest; organizing, monitoring and evaluating extension activities; developing training programs; and organization and evaluating capacity building cycles.
3. Production and dissemination of extension materials (*e.g.* audiovisual media) and dissemination of information on all types of media.
4. Capitalization, processing and dissemination of information: creation of an agricultural data bank; processing and disseminating information.

Source: Korichi (2010).

The primary extension method is through demos, workshops and field days followed by farmer field visits. Radio programs are the main means of ICT (IFPRI, 2012). The Ministry of Agriculture has a network of demonstration units preferentially located at farmer level and in the 69 stations of the centres and institutions disseminated in Algeria (Korichi, 2010).
National agricultural extension services through their regional stations located in all the provinces (*i.e. Wilayas*) of the country implement various extension activities for farmers on different topics according to crop and livestock species that characterize each region. Extension at *wilaya* level is ensured by the collaboration of various agricultural institutions, such as chambers of agriculture and technical institutes (Laoubi *et al*., 2010).
In order to better coordinate the main agricultural research and extension programs towards farmers was launched in 2009 by INVA a programme called PRCHAT1 (*Programme de Renforcement des Capacites Humaines et d'Assistance Technique* / Programme for Strengthening Human Capacities and Technical Assistance). The main aim of this program is to improve and enhance human capacities and technical assistance regarding different programs of rural and agricultural renewal through: expanding the intervention capacity of support and development structures (INRA, INPV, ITGC, ITCMI, etc.); strengthening coordination and consultation mechanisms; and bringing technical structures to local level. The program also aims at: correcting the gaps of human resources (both technical and administrative) in implementation, training and development activities; improving the capacity of central and decentralized administrative structures for a permanent and effective follow-up of PREAR; improving the capacity of research structures and development of technical guidance; setting up a network of demonstration units; setting up a network of farmer field schools; mobilizing competencies and infrastructure; and intensifying programs of mass extension. Several decrees have been issued by the Ministry of Agriculture related to

extension and training actions funding such as the decrees n°916 to 920/2008. An important budget was dedicated to these programs towards farmers, rural population and technical agents involved in the PREAR. The extension strategy and operational plan for the five-year period, 2010-2014, focuses on participatory planning, intensive training of extension workers, development of multi-media materials, and processes for managing, monitoring and evaluating actions. These activities are intended to be applied by extension staff to support the policies, strategies and priority programs of agricultural and rural development.
The balance of PRCHAT1 activities (2010-2014), showed a significant dynamic in extension area. According to DFRV, 284,935 people were trained, including 116,823 technicians, 162,413 farmers and herders, and 5700 other stakeholders (*e.g.* agricultural and rural development projects managers) (Table 1).

Table 1. Balance of activities carried out in the framework of PRCHAT1, period 2010-2014.

Activity	Beneficiaries	
Training	284,935 trainees including	116,823 technicians
		162,413 farmers & breeders
		5700 other actors (*e.g.* project
Extension	1,033,789 persons including	911,547 farmers & breeders
		72,923 technicians
		49,319 young people dealing with
	297 projects including 207 national projects (National Program of	
Remark: Actions and projects dealt with all topics mainly strategic ones		

Source: DFRV (2015)

Since January 2015, the DFRV launched PRCHAT2. The new program is a continuation of PRCHAT1. Its aims are: access to quality extension by all stakeholders; specifying the place and role of each actor in the extension system; creation of a training engineering unit to improve training offer quality; institutionalisation of more comprehensive training agreements; introduction of monitoring / evaluation tools focused on training quality; and mobilization of additional financial resources.
The results of the first quarter of 2015 encompass the advent of Multiannual Programme (2015-2019) and the starting of PRCHAT2 program. The number of trainees in the first quarter of 2015 had a decline compared to the first quarter of 2014 (Table 2). This is due to the qualitative approach adopted in PRCHAT2 program: group training has been limited, for pedagogical and organizational reasons, for a better impact on trainees.

Table 2. Global population trained in Algeria and abroad in the framework of PRCHAT2 program; first quarter of 2015.

Global population trained in Algeria			
Beneficiaries	Training (1)	Other actions (2)	Total
Agents	3018	1528	4546
Farmers / breeders	10 941	107	11048
TOTAL	13 959	1635	15594
(1) Training actions lasting at least two days (2) *Seminars, workshops...*			
Global population trained abroad			
Beneficiaries		Number	
Technicians		49	

Source: DFRV (2015)

Training and extension sessions were organised in all the 48 Algerian *wilayas*, especially in extension and demonstration centres and farms, and dealt with different agricultural sectors and topics (Table 3).

Table 3. Evolution of extension sessions number by production sector between the first quarter of 2014 and the first quarter of 2015.

	Number of sessions	
Topic	**First quarter 2014**	**First quarter 2015**
Intensification of cereals and legumes	384	498
Development of olive growing	156	179
Seeds and crop protection	115	112
Fruit trees	88	108
Intensification of potato	94	107
Water-saving irrigation systems	81	92
Livestock and animal health protection	67	74
Milk production	27	17
"Creating new farms" and "Youth employment" measures	128	95

Source: DFRV (2015).

Many partners are involved in the implementation of training actions:
- Institutions of MADR: Direction of Agricultural Services, chambers of agriculture, training centres and institutions, technical institutes and research centres, etc.
- Other institutions *e.g.* training institutions of the Ministry of Education and Professional Training;
- Foreign institutions and organizations: Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD), International Atomic Energy Agency (IAEA), DG SANCO, CIHEAM, EFSA, ICARDA, UNDP...

Moreover, in the framework of the implementation of a three-years, 2015-2017, institutional partnership (PROFAS - *Programme Algéro-Français de Formations Supérieures*) between DFRV (MADR) and the French Ministry of Agriculture for 2015 many actions have been carried out: support for the establishment of a national agricultural training engineering unit; support for the redefinition of the role of training institutions; exchange of experience between Algerian and French technical institutes; support for the establishment of a platform for inter-ministerial dialogue on training in and qualification of the agricultural sector.

Conclusion

Algeria has developed an important support system including measures to promote professional organizations and institutions and to support agricultural production. However, nowadays, Algerian farmers are increasingly asked to respond to the challenges facing them. The profound changes occurring in the agricultural environment also require them to have more knowledge. The availability of effective and efficient extension is highly desired.

Due of its status, the national system of agricultural extension led by INVA, und under the institutional and political supervision and guidance of DFRV at the Ministry of Agriculture, could be an answer to the needs of pooling local resources and opening to international networks. This is related to the need to restructure the system, from the organizational and functional points of view, and to strengthen its human resources by mobilizing sectoral competences and external resources. Analysis of informational needs of farmers should

constitute the basis for building the extension offer. The new agricultural training and extension programs (PRCHAT1 and PRCHAT2) are going in this direction and should allow strengthening the agricultural extension system and improving its performance so that it can appropriately contribute to the sustainable development of Algerian agriculture and rural areas.

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DETERMINANTS OF ACCESS TO AGRICULTURAL CREDITS FOR SMALL SCALE FARMERS IN THE SOUTHERN PROVINCE OF RWANDA

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Abstract

This study investigated the determinants of financial inclusion for small scale farmers by focusing on the access to formal and informal agricultural credits in the Southern Province of Rwanda. Data were collected from 310 farmers from Huye and Nyamagabe districts through an open-ended structured questionnaire and analysed using the binary logistic regression method. The major findings, on one hand, showed that among the requested and approved agricultural loans, 87.2% and 12.7% were from informal and formal financial institutions respectively. Among those who accessed agricultural credits, 59.7% were from Huye and 40.3% from Nyamagabe. On the other hand, it was revealed that the household characteristics and the community attributes are the most important determinants. Those household characteristics include household income and expenditure, Ubudehe socio-economic category of the household, off-farm employment and the size of the land owned by the household whereas community attributes involve residence area, transport and informal financial services availability. Regarding farmer characteristics, the education level was found to be the only factor affecting the smallholder farmers' access to agricultural credit. In light of the findings, it was recommended to conduct sensitization sessions focusing on the importance of agricultural credits for smallholder farmers especially in the areas with high level of poverty. In addition, there is need to work on alleviating the formal agricultural credits access barriers, and to conduct a study on the dynamics of informal and formal agricultural credits up take and usage by smallholder farmers to explore all dimensions of financial inclusion in the study area.

Keywords: *agricultural credit, small scale farmers, Southern Province, Rwanda*

Introduction

The economy of Rwanda is highly depending on agriculture which employs 87.3% of economically active population and contributes up to 36% in the GDP (Gisaro, 2013). More than 70% of its adult population live in rural areas (NISR, 2012a) and rely mostly on agriculture for their daily livelihoods. Given the importance of agriculture to national economy, the raising of agricultural productivity and farmer’s income remains the priority of the Rwandan government. The agricultural credit through microfinance institutions has been selected along with other mechanisms to achieve the main goal of transforming and modernizing the agricultural sector highlighted in different agriculture-related programs and strategies. The importance of credit for rural development is even more pronounced for developing countries with largely subsistence farming systems such as Rwanda (Muhongayire *et al.*, 2013), where, there are two major sources of agricultural credit namely formal institution and informal institution sources (NISR, 2012a). The first comprises development and commercial banks, microfinance institutions, and Saving and Credit Cooperatives (SACCOs) while the latter is made by internal value chain financing agents, agricultural

cooperatives, farmers' associations, tontines, loans clubs from Non-Government Organizations, friends and neighbors.

In the literature, it has been shown that agricultural credit is a tool to expand agricultural production (Balogun and Yusuf, 2011) and to promote the standard of living by breaking vicious cycle of poverty of small scale farmers (Ayegba and Ikani, 2013). It serves to improve the household welfare by increasing the total consumption levels and impacting positively the demand for children's health care and education, as well as leisure (Armendariz and Morduch, 2005). Agricultural credit is also seen as an important financial support that a small farmer can get in order to bridge the gap between his income and expenditure (Khan *et al.*, 2011).

In rural areas, the availability of agricultural credit and financial services is perceived as a critical matter but the access to these financial services by rural farmers is another one. This, because their availability does not guarantee their accessibility. As argued by Duy *et al.* (2012), the success of credit provision for poverty reduction depends on the possible access by poor households to credit-providing institutions. According to De Klerk *et al.* (2013), in sub-Saharan Africa, where most people still live in rural areas and agriculture is the mainstay of the rural economy, access to financial services of all kinds appears still to be poor. In this line, Anyanwu (2004) states that collateral, credit rationing, preference for high income clients and large loans, bureaucratic and lengthy procedures of providing loan in the formal sector keep poor people outside the boundary of the formal sector financial institutions in developing countries. According to Swinnen and Gow (1999), for most banks, financing agriculture is a high risk activity because of low profitability in the sector. As asserted by DID (2010), the other problem of lack of access is related to the fact that the farmer is faced with financing needs related to his family. The author also argues that women face significant family burdens related to child rearing, healthcare, clothing and other basic family needs, and this situation often leads them to exhibit greater aversion to risk and, therefore, be less inclined to use credit as a development tool.

In Rwanda, besides the fact that some of rural credits are not adapted to the activities and profile of farmers, a number of factors – including lack of awareness of rural farmers regarding rural credits availability and utility, fearness to take credit, difficulty to meet eligibility criteria for farmers to access bank credit, high interest rate on bank credits, physical access-distance to formal financial institutions, poverty and other deprivations – have been identified as limiting the access of rural farmers to credits (MINAGRI, 2009; NISR, 2012a). The Government of Rwanda, through various mechanisms such as setting up projects, task forces, funds and local saving and credit schemes as SACCOs in each administrative sector tried to find out solutions to overcome the aforementioned hindrances. Despite all the efforts made, Muhongayire *et al.* (2013) affirm that, access to formal credit remains steadily low even as the national economy is considerably growing. A study by NISR (2012a) reveals that in 2012, 71.9% of adult population were financially included (or have had access to formal and/or informal financial products) but only 7.4% applied for agricultural credit and 90.5% of them were approved. Agricultural inputs are less affordable to farmers because of lack of domestic sources of fertilizer and high cost of pesticide, while most farmers are poor and lack access to credit to finance inputs (IPAR, 2009). Consequently, agricultural productivity is still low and for some food crops such as maize, rice and other selected priority crops, the country relies on imports and many rural households are living in poverty with 44.9% in poverty and 24.1% in extreme poverty (NISR, 2012b).

While it has been shown that the level of investment in agricultural activities is correlated with agricultural productivity which goes together with improving food security and reducing poverty (Khan *et al.*, 2011; Zeller *et al.*, 1997), the following concern arises in the context of

rural areas of Rwanda where, according to NISR(2012b), smallholder farmers still face low productivity and poor living conditions: What are the factors affecting the access to both formal and informal agricultural credits for smallholder farmers?
Studies (for example, Sebakambwe, 2012; Muhongayire *et al.*, 2013) have documented the issue of rural credit in Rwanda but none has been conducted to study the accessibility of agricultural credits in Southern Rwanda from the point of view of the demand side. Therefore, this study seeks to identify the determinants of access to formal and informal agricultural credits for smallholder farmers in the Southern Province of Rwanda.

Materials and Methods

This study was carried out in Huye and Nyamagabe districts of the Southern Province of Rwanda, a purposively selected study area. The choice of this area was motivated by the highest level of poverty prevailing in this Province (56.5%), and to enhance the situation understanding, the districts of Huye and Nyamagabe were selected as they are respectively least poor and poorest in this Province (NISR, 2012b). The study used a sample of 310 farmers determined using the Cochran (1963) sampling formula based on the financial access strand of 71.9% in Rwanda (NISR, 2012a). Sampling frames were obtained from heads of six agricultural cooperatives selected in the two districts (three in each). A simple random selection technique with probability proportional to size was used to obtain the number of interviewed farmers in each of the cooperatives. The data collection was performed from January to March 2015 and used an open-ended structured questionnaire highlighting the main features pertaining to the objective of the study. Given that our model has a dichotomous dependent variable with two values, 1 (when the farmer is having access to agricultural credit) or 0 (otherwise), data were analysed using the binomial logistic regression method following Hosmer and Lemeshew (1989). The set of explanatory variables is made by continuous and categorical/dichotomous variables on the community and institutional characteristics, socioeconomic and demographic situation, land and dwelling endowment among others.

Results and Discussions

The descriptive analysis reveals that among the requested and approved agricultural loans, 87.2% were from informal sources while only 12.7% were from formal financial institutions. The study shows that 59.7% of those who accessed agricultural credits were from Huye district and 40.3% from Nyamagabe district.

Regarding Ubudehe socio-economic categories namely first category (people in abject poverty), second category (very poor people), third category (poor people), fourth category (resourceful poor people), fifth category (food rich people) and the sixth category (money rich people) (NISR, 2012c), the results showed that 37.5% of farmers from Nyamagabe district are in the first and second socio-economic categories. Those from Huye district in those very same first and second categories accounted for 32.3%. Among the interviewed farmers, 62.6% and 66.5%, respectively, from Nyamagabe and Huye districts are counted in the third socio-economic categories. All interviewed farmers from Nyamagabe district belong only to the first three categories whereas 1.3% of the respondents from Huye district are in the fourth category. None of the sampled farmers is in the fifth and sixth socio-economic categories.

Concerning the factors affecting the access to agricultural credits, the results of the logit estimation are presented in Table 1.

Table 1. Binomial Logistic Regression model: Estimation results

Variable	Coeff. (β)	Stand. Error	p-value	Exp (β)
Constant	-4.373	1.952	0.025	0.013
Individual level				
Gender	-0.028	0.379	0.941	0.972
Marital status	0.423	0.450	0.347	1.527
Education level	0.403	0.237	0.049*	0.648
Agricultural training	0.431	0.695	0.535	1.539
Awareness about microfinance institutions	0.138	0.722	0.848	1.148
Farming activities recording	0.499	0.455	0.272	1.648
Household level				
House ownership	0.438	0.833	0.599	1.550
Livestock ownership	-0.116	0.403	0.774	0.890
Income	-1.046	0.371	0.005**	0.352
Land size (in acres)	0.014	0.007	0.037*	0.986
Farm profitability	-0.180	0.433	0.678	0.836
Ubudehe socio-economic category	0.415	0.287	0.047*	1.514
Number of household members	0.029	0.089	0.741	1.030
Household monthly expenditure	-0.924	0.440	0.036*	0.397
Off-farm employment	2.033	1.230	0.041*	7.636
Community and Institutional level				
Area of residence	0.612	0.475	0.049*	1.844
Roads availability	0.649	0.437	0.137	1.914
Distance to microfinance institutions	0.082	0.474	0.863	1.085
Transport availability	0.889	0.420	0.034*	2.433
Availability of agriculture-related information	0.846	0.495	0.088	2.330
Informal financial services availability	3.833	1.514	0.009**	46.21
Length of the loan procedures in formal institutions	-0.841	0.685	0.219	0.431

(*): significant at 5% and (**): significant at 1%

-2Loglikelihood=226.345 Cox and Snell R^2=0.280 Nagelkerke R^2=0.405
Hosmer and Lemeshow test: Chi-square=6.768, p-value=0.562

The study reveals that the level of income of the household head and informal financial services availability in the neighborhood are the most significant variables. The availability of informal financial services increases the probability of having access to agricultural credit by 383.3% whereas the level of income of the household decreases the probability of accessing agricultural credit by 104.6%. This empirical finding supports the assertion that higher income households may be less risk vulnerable and have less demand for credit because they have enough capital from previous earnings (Nunung *et al.*, 2005). The significantly positive coefficient of the size of the land owned by the household variable in the model suggests that the size of the land increases a farmer’s likelihood of having access to agricultural credit.

The results show that being a resident of Huye district increases the probability of having access to agricultural credit by 61.2%. Rural farmer's access to agricultural credit is also positively affected by off-farm employment in the household and transport availability in the community. High level of education of the farmer and Ubudehe socio-economic category of

the household are also positively significant in determining the probability of having access to agricultural credit. The empirical model reveals that an increase in one level among Ubudehe socio-economic categories multiplies the likelihood of having access to agricultural credit by 1.5 and the level of education of the farmer increases the probability of having access to agricultural credit by 40.3%. This appears very intuitive and evident as a literate and/or a wealthier farmer is likely to have a better understanding and then be opened for financial products and services than an illiterate and/or poor farmer who is less likely to manage his business with success. The results show that when the household monthly expenditure is perceived as high compared to its monthly income, the probability of having access to agricultural credit decreases by 92.4%. This may be explained by the fact that when the small scale farmer faces financing needs related to his family (consumption expenditure, education, health and leisure) and to his farm (purchasing inputs, rent for land, cost of post-harvest technology, …) which require an amount greater than the income from his farm, this may lead to an increase in the risk of his ability to repay the loan and then, to less demand for agricultural credit.

However, the estimated coefficients on gender, house ownership, livestock ownership, distance from home to micro finance institutions, time taken by the loan procedures in formal institutions, farm profitability and the number of the household members are not statistically significant and were not found to be factors affecting the access to agricultural credits. These results are not in line with those found in the literature (for example, Sebakambwe, 2012; Anyanwu, 2004; Swinnen and Gow, 1999) in regards to rural credits. This may find its explanation in the fact that in rural areas, where the level of poverty is high with subsistence agriculture, farmers mostly rely on agriculture-related activities funding from informal financial institutions which are mostly found in the neighborhood, and where the gender of the applicant, the collateral and the guarantor are not among the main requirements.

Conclusion

This empirical study investigated the determinants of access to formal and informal agricultural credit by smallholder farmers from the Southern Province of Rwanda. The results revealed that the household characteristics and the community attributes are the most important factors, affecting the farmers' access to agricultural credit. The transport and informal financial services availability at community level have been found as the most significant determinants of agricultural credit access. The residence area, the level of education of the farmer, the Ubudehe socio-economic status of the household and off-farm employment in the household are also positively associated with the likelihood of having access to agricultural credit. The level of household income, the size of the land owned by the household and the level of household monthly expenditure are negatively affecting the farmers' access to agricultural credit. In light of the findings, the following recommendations emerge: the sensitization sessions focusing on the importance of agricultural credits in enhancing the smallholder farmers' operating conditions are needed especially in the areas with high level of poverty; for formal agricultural credits, policymakers should work on alleviating the access barriers; and, to explore the three dimensions of financial inclusion for small scale farmers in this area, a study on the dynamics of informal and formal agricultural credits up take and usage is necessary.

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TESTING OF A TECHNICAL-ECONOMIC REFERENTIAL IN IRRIGATED AGRICULTURE IN BURKINA FASO

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Abstract

The practice of irrigated agriculture in Burkina Faso still suffers by the weak control of technical-economic parameters for an agricultural production capable to ensure sufficient income to irrigating producers. Therefore, the efficient valuation and profitability of irrigated infrastructures remain mitigated. To overcome this deficiency, a study for the development of a technical-economic referential of irrigated crops is an alternative to produce information with a view to orient the irrigated agricultural holdings in Burkina Faso on irrigated production methods. This study is conducted in this perspective.

The study is based on panel data collected through an agricultural accounting observatory of irrigated crops in sixteen irrigated perimeters during three agricultural seasons, each consisted of a dry season and a rainy season. It performs an analysis of gross margins, cost prices of irrigated crops and capital income and labor of irrigated agricultural holdings.

In the analytical process of economic parameters, purpose of the present article, two approaches are used: the first approach is based on the comparison of study parameters according to the crops grown, while the second is a comparison by type of irrigated schemes.

Keywords : *technical-economic referential, panel data, irrigated crop, agricultural holding.*

Introduction

Agriculture in Burkina Faso is highly dependent on weather conditions. Seventy-five percent of agricultural production in the country is rainfed. Since the drought of the 1970s, the development of irrigated agriculture became essential to improve agricultural production and secure food resources of households.

In spite of efforts to increase investment in developing irrigated areas, the results in terms of valorization, however, remain mixed. For instance, in large areas, they are between 79 and 100% during the wet season and 55 to 100% during the dry season. Also the yields of major irrigated cereal crops globally are weak on the average: 3.5 to 4.5 t/ha both in the rainy and dry seasons for the rice and 2.5 to 3 t/ha during the two seasons for maize) (MAH, 2011).

Despite the difficulties of developing irrigated areas, their profitability remains low. Indeed, the internal rate of return hardly exceeds 20%, regardless of the type of development.

In short, the practice of irrigated agriculture in Burkina Faso still suffers from the weak control of technical and economic parameters for an efficient agricultural production that can ensure food security and fight against poverty.

To address this concern, the study of the development of a technical and economic repository for irrigated crops in Burkina Faso turned out such as an alternative tool to produce information to guide and properly improve the practices of irrigated crops.

Worldwide, agriculture repositories are established through methods mainly focused on the observation of farming practices over time in order to establish a diagnosis for better guide. For instance, development of technical and economic repository of Guyana crops and

livestock production (CGERG, 2002) based on observations of farming practices that lasted 4 years helped out (among other economic indicators) average prices, gross margins and technical data related to crop production. The same process was used in a study in Mali to establish a technical and economic database of the Diffusion of Irrigation and Production Technologies (RTE/DTIP). Essential information sought concerned the determination of the main production areas of horticultural and arboricultural crops, the farmers operating accounts, the crops yields, the labor's values.
The approach for the establishment of agricultural frameworks for improved efficiency in production was applied in South Vietnam for rice, in Wallonia for the evaluation of performance and profitability in agriculture; this is in order to make available to the agricultural world tools that improve activities performance. For these studies, a common thread emerges: it is the implementation of a collection method based on 3 years (minimum) of observations. According to Lebailly et al. (2000), the observatory based on a semi-permanent sample of holdings, provides food for thought on agricultural policies to be formulated. Indeed, agricultural policies determine widely the economic situation of the farmers and, therefore their strategic reasoning (Ansaloni and Fouilleux, 2006). For most of the cited studies, the analysis consisted of comparing parameters and information production for the orientation of policy makers and practitioners. Pierrot (1990) gives evidence of comparative approach by asserting that technical-economic research on the study of functioning of the exploitations allows building a relevant device to compare their performances with a structured reference table. This approach facilitates the formulation of diagnostics and adapted agricultural advice.
As part of this paper, our choice fell on corn, rice and onions because of the strong momentum observed around these crops, both technically and economically (according to statistics).

Materials and methods

In the case of Burkina Faso, the study for the development of technical and economic standard was driven through the establishment of an observatory of irrigated agriculture accounts for the entire national territory. It consisted of conducting surveys during agricultural seasons (both dry and rainy season) from 2009 to 2012. 16 irrigated areas were selected according to criteria relating to the agro-climatic zone, the size and the schemes of water pumping system. At each site, 10 randomly selected farmers were followed for all crops grown in irrigated and rainfed.

The collected data have focused on the structure and activities of monitored farms, the crops acreage (rainfed and irrigated), the production system, the activities of the production ways, the means used and the acquisition modes, the fees, the yields, the post-harvest activities, products and different destinations (consumption, market).

The compilation of the data collected was carried out using a database created with MS-Access. After encoding data, they have been cleaned (using statistical methods and additional investigation) to remove missing data and outliers. The rate of these outliers and missing data is globally estimated at 4%.

To ensure the quality of the collected data, a method of replacing missing data or outliers was used (by using the mean, median or mode).

However, given that extreme values or outliers can influence the average and make it unstable, median observations were used.

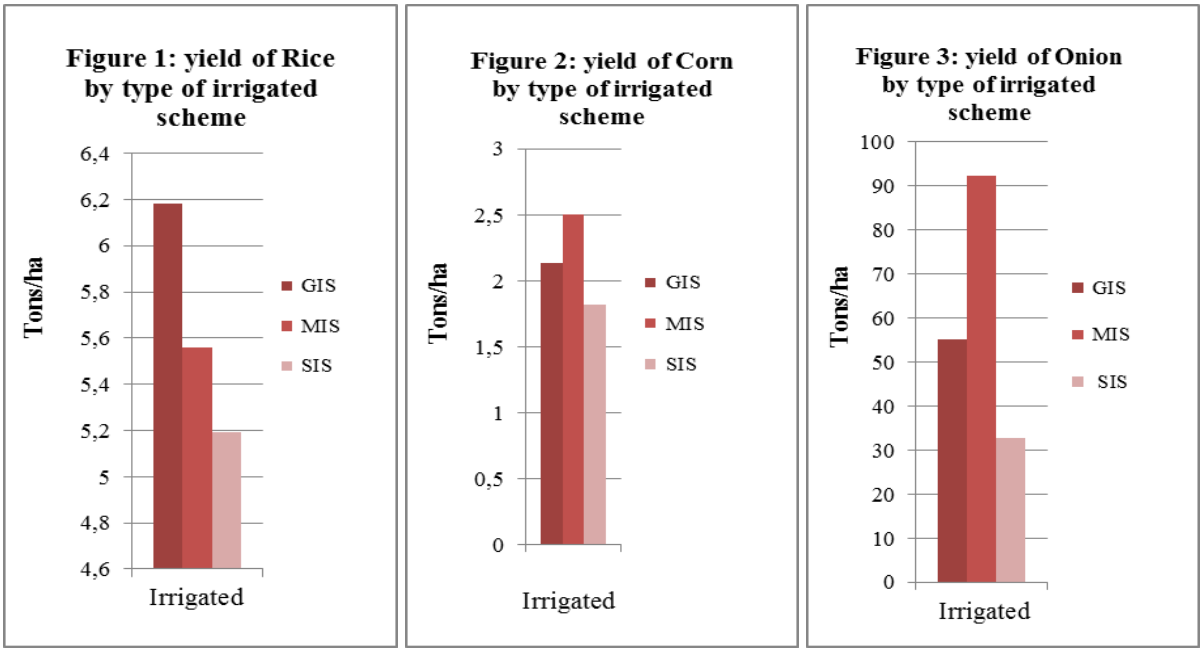
Also, procedures have been incorporated into the database for the calculation of economic indicators such as gross margins, cost, and labor income of irrigated farms.

From the database, the data taking into account only the producers regularly monitored during the period were used for the analyses. SPSS and MS-Excel were used for data processing and the calculation of economic parameters. The analyses consisted of comparing different crops according to the production system and the typology of irrigated schemes.

One difficulty in the constitution of the base sample was the high mobility of producers. Therefore in this study, only regularly monitored producers were selected.

Results and discussion

The results show some technical indicators for crops like rice, corn and onion. As part of the technical indicators, the figures below show the performance of crops depending on the irrigated scheme[15]: great irrigated scheme (GIS), medium irrigated scheme (MIS) and small irrigated scheme (SIS).

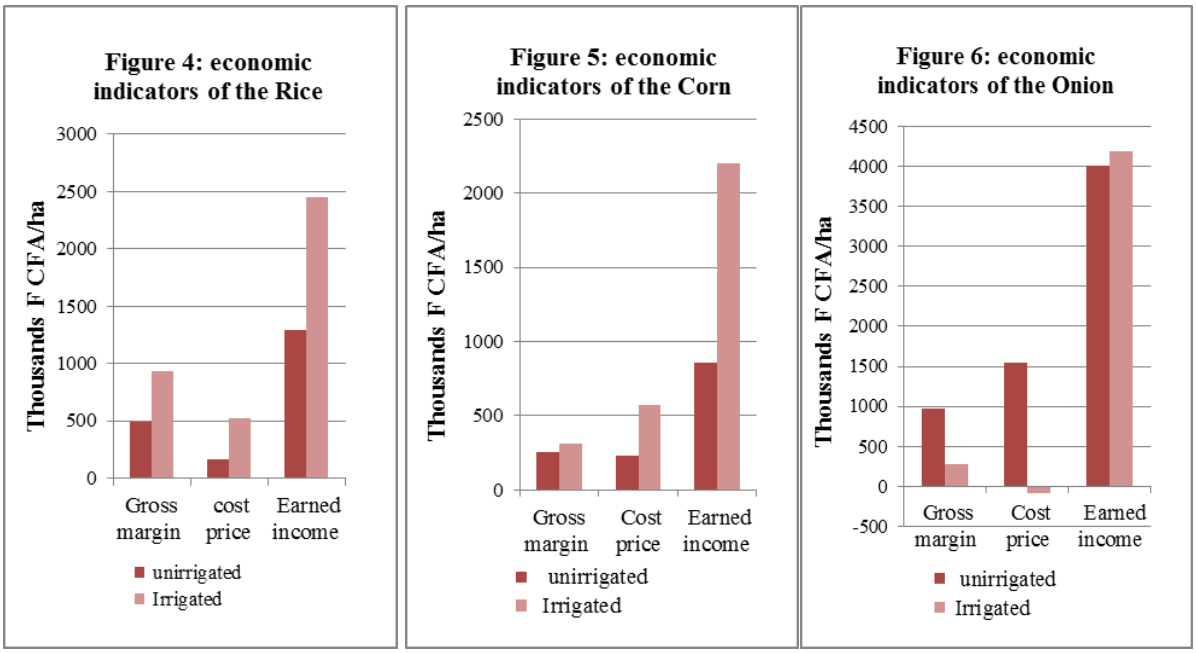


Yield of irrigated rice is higher in large irrigated areas and is around 6 tons per hectare (figure 1). This is probably due to the use of modern means and compliance with good agricultural practices in the production of large-scale rice, unlike small and medium areas in which production is still of family type.

Corn and onion production on medium area perimeters, have the highest yields (figures 2 and 3), which can be explained by the fact that these irrigated crops are generally produced on medium areas. Their productions controlled over large areas are extremely rare (or almost nonexistent for onion).

[15] GIS : area ≥ 100 ha ; MIS : 20 ha ≤ area < 100 ha; SIS : area < 20 ha

Concerning the economic indicators, the following figures show the gross margins, cost, and the income of the studied crops.



In light of these economic indicators for corn and rice, we have the same trends for the three studied indicators. The gross margins, cost and labor incomes for these two crops are higher for irrigated agriculture. This is justified by the fact that irrigated agriculture has more charges than unirrigated agriculture. These cereal crops irrigated are also more productive than those cultivated in pluvial. Figures 4 and 5 illustrate this fact.

The special case of onion illustrated by the figure 6 could be explained by the fact that onion is grown mainly in the dry season (irrigated) and especially on small areas without the introduction of large means. This makes these productions very vulnerable.

Conclusion

The establishment of the technical and economic repository on irrigated agriculture based on farmers practice in Burkina Faso is in a process of producing relevant information to help orient the stakeholders in the practice of irrigation.

By establishing the importance of irrigated production through the production of these few indicators, the study is looking at the effects of irrigation on agricultural production in terms of efficiency. With climate change, water is becoming increasingly scarce over time. We believe that a good control of irrigation systems combined with good agricultural practices should lead to increased productivity and yields, and improve the living standards of the producers. The next step will be a complete analysis of technical and economic parameters of other cultures.

In order to improve the living standards of producers, Burkina Faso could guide and support irrigated rice farmers to better optimize the means of production and move towards professionalization of their activity. In a context of quest of food security, the fight against poverty and climate change, agricultural consultancy in the area of irrigation should move towards the practice of irrigated crops of high productivity and high value. As such, the production of corn and onion, profitable crops, should be encouraged in areas equipped with water control.

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SYSTEMIC ANALYSIS OF TECHNICAL EFFICIENCY OF IRRIGATED FARMS IN BURKINA FASO

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Abstract

In this study, the "Data Envelopment Analysis" (DEA) and a systemic approach is applied to assess technical efficiency of irrigated farms across 13 regions of Burkina-Faso. Ordinary Least Squares method is used to identify technical efficiency determinants.

Data were collected during three agricultural campaigns, from 125 farms in three regions differentiated by three main parameters: (1) zones irrigation capacities; (2) two types of irrigation and; (3) two different modes of water drainage.

We seek to establish a causal link between farms efficiency and household socio-demographic factors and the above mentioned endogenous parameters. Over the survey entire period and whatever the parameters are; the average inefficiency scores (6.33±5.71) is high. By improving their resource allocation strategies, irrigated farms would be able to reduce their pure technical inefficiency scores (5.06 ± 4.98) and; thus quintupled their production.

Such inefficiency scores did not significantly vary with zones irrigation capacity. But farms using pumping for irrigation are relatively more efficient. Farms are more efficient in dry season than in rainy season. Invariably, technical efficiency is negatively correlated with the farms size. Optimal acreage allows increasing production by 30%. Also, we notice that farms efficiency levels are significantly influenced by household size, labour employability, land tenure and crop diversification.

Keywords: *irrigated farms, technical efficiency, DEA, systemic analysis.*

Introduction

In Sub-Sahara Africa (SSA), specifically in Burkina-Faso, production systems are highly affected by rains variability. The reduction of pluviometric gradient is a clue of climate change; and these modifications threaten the people's livelihoods (Sivakumar, 1998).

Therefore policy makers decided to develop strategies and programs to enhance rural population resilience to livelihoods vulnerability and food insecurity. And one of their focuses is water irrigation systems as a main tool to improve farming productivity (Rosegrant and Cline, 2003; Diouf, 2008; CILSS, 2010).

In Burkina-Faso, the implementation of these policies were supported by World Bank and other National Funds; and it permitted to increase the share of irrigated crops in the agricultural Gross Domestic Product (GDP) from 2.12% in 2005 to 8.33% in 2010. Regarding the National GDP, it increased from 1.9% in 2006 to 3.1% in 2008 (MAH, 2011).

In west-Africa to date, irrigation systems scaled at farm level "small irrigation" is the most common. Due to its low investment cost, it is accessible to small farmers and vulnerable

groups. But (MAHRH, 2004) noticed that, besides the availability of water and land; results of empirical surveys on small scale irrigation systems efficiency vary greatly.
Firstly, there are important fluctuations of acreages and yields (decreasing yields) over years; with no diagnosis to determine the real causes (MAH, 2011). These fluctuations lead to difficulties to plan and identify the optimal acreage of a profitable farm. The factual approach gives realistic information and captures better farms production systems complexity.
On the other side, it is noticed an apparent inefficiency of irrigated farms whatever the production season. Previous studies on irrigated crops such as rice and leguminous have shown that farmers technical efficiency is low (Kaboré and Taondyandé, 2007; CAPES, 2011). These results are confirmed in SSA countries by Audibert and al. (2003) and Nuama (2010) in Ivory Coast; Adégbidi (2003) in Benin; Diagne and al. (2013) in Senegal.
Despite the weaknesses of irrigated agriculture in SSA, stakeholders still interested to support and fund the sector.
This contrasted situation leads to wonder on how and which strategies to improve irrigated farms' technical efficiency; regarding its importance towards food security and poverty reduction. Before answering this question, we need to diagnose small scale irrigated related to production efficiency.
To provide some insights to these questions, this study compares irrigated farms technical efficiency and identifies the factors influencing this efficiency.
Specifically, the paper will evolve as following:
comparative analysis of irrigated farms technical efficiency with parameters such as: the regions' irrigation capacities; national typology of irrigated schemes; water uses patterns and agricultural seasons;
irrigated farms technical efficiency scores determinants' identification.

Materials and methods

The paper is drawn on systemic modeling theory, which was proofed to fit agricultural farms characterization regarding their technical and economic features (Adégbidi, 2003; Djagni, 2007). According to Abatania (2012), multi-purposes phenomenon cannot be captured using the classical approach, which does not allow comparison between actions; as in the case of farming production. While, the systemic modeling refers to the farms' scale analyses and permits to characterize and compare performances of associated production systems.
In this study, irrigated farm is defined as a Decision Making Unit (DMU) using irrigation for agricultural production during the dry or the rainy season. Each farm is distinguished by its irrigation capacity, the type of irrigated scheme and water collection methods used.
Due to vulnerability and risk aversion, a DMU can diversify its activities by combining crop production during the rainy season and others types of activities (animal breeding, services, gold panning, etc.). A DMU can integrate several production systems (irrigated or non-irrigated) which can be located in different geographical areas (equipped plots or non-equipped plots).
For a specified DMU, there are interactions between dry season activities and rainy season activities, which lead to inseparability of allocation resources for production decisions. Then, the systemic approach allows assessing the impact of the rainy season production and other types of production on the dry season irrigated production; and to capture the system inner performance (Figure1).

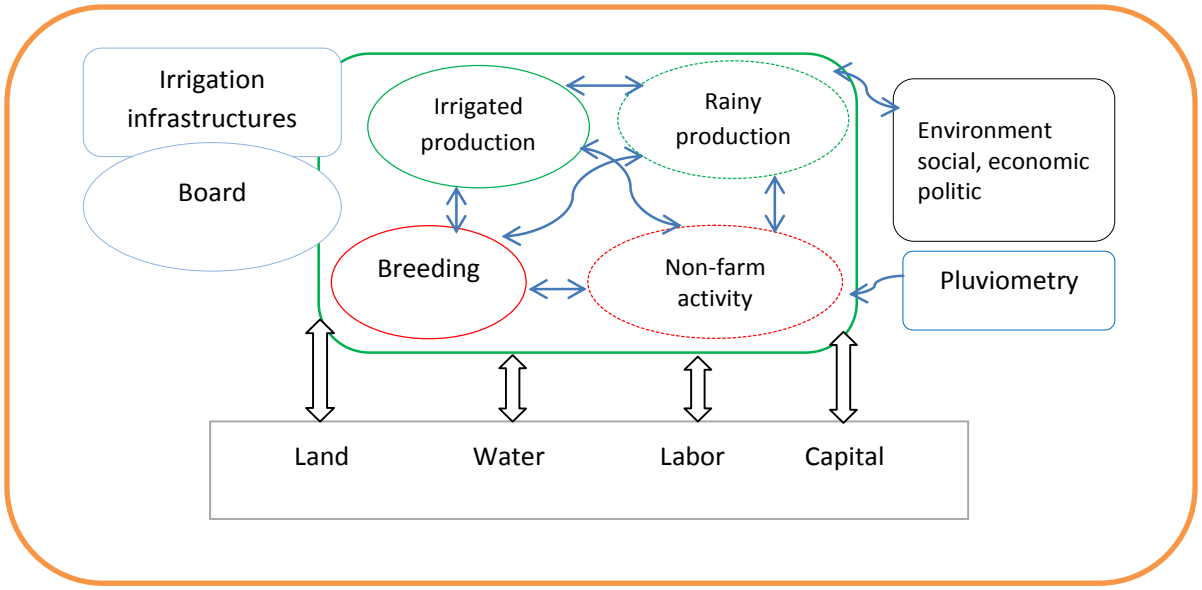

Figure 1: Theoretical framework
Source : Authors' representation

In this study the authors used the DEA method to assess irrigated farm technical efficiency and scale efficiency. We adopt the output oriented function because agricultural policies used in Burkina-Faso target yields increase instead of inputs reduction [5]. Therefore, both methods BCC and CCR may give realistic information under constant return to scale.

At the start of the process of building the study sample, we had the idea to develop the major work done at the national level in irrigated agriculture.
Starting from this idea, the option was taken to consider zoning, which divides the country into three main areas; having major irrigation potential.
Each area includes irrigated areas classified according to their size. In addition to the irrigated area size, which is a factor of discrimination; we considered the method used for conveying the water (with or without pumping).
This last parameter can be considered de facto as an important element of farm performance differentiation, particularly in terms of production costs and hence production results.
A total of 16 irrigated schemes were selected for sampling. On each irrigation scheme, 10 farms were selected randomly.
A sample of 160 farms was then constituted for monitoring. For various reasons (death, economic and land mobility of farmers, etc.), only 125 irrigated farms have been assiduously followed during three years. These farms are scattered across the country and represent several facets of irrigated agriculture.
The studied farms are characterized by production systems (irrigated and / or rainfed), which are characterized by some crop diversification. Diversification levels calculated from the transformed Herfindahl index (THI) were 81.8 % (for 23 crops) and 77.3% (for 19 crops), during the rainy season and dry season, respectively. A total of 25 different crops (cereals, horticultural crops, tubers) were counted.
Data were collected through interviews and agricultural accounts tracking methods. The observations lasted three (03) agricultural campaigns: from 2009 to 2012. Each agricultural campaign includes two cropping seasons : a rainy season that lasts from may to september and a dry season, from october to april. Panel data have been collected and includes : information about socio-demographic characteristics of the household's head (gender, age, marital status, educational level and origin of the manager ; size and number of household assets, land

tenure) ; farm resources and type of operation in the production process; techno-economic and synthetic accounting data (agricultural yield, gross margin, operating income, value added, cost, operating costs, etc.).
Usually, the used factors are: land, inputs and labor. In our case, the choice of variables was made according to the literature and our empirical knowledge of irrigated production practices.
Accounting for observations criteria and the convexity assumption imposed on the production frontier, our analyses focused ultimately on 321 DMUs.
The model output is an aggregate indicator that represents the sum of the values at market prices (FCFA) of all vegetable productions in the dry and rainy seasons. The inputs are: (1) total acreage (Ha); (2) total value of the seeds (FCFA); (3) total amount of fertilizer (NPK) (kg); (4) total quantity of fertilizer (Urea) (kg); (5) amount of labor used for all cultures (Man/Day); (6) capital in monetary values (FCFA): depreciation of equipment, agricultural and irrigation equipment, and buildings.

Results and discussion

3.1. Global Technical Inefficiency scores

Descriptive statistics of technical inefficiency scores of CCR or CRS models are presented in Table 1. During the period of research, farms Global Technical Inefficiency (GTIn) did not vary significantly. Regardless of cropping seasons, the mean score for global technical inefficiency, after bias correction by the method of (Simar and Wilson, 1998), is 6.33 (± 5.71). The score dispersion range includes less than 24 % of DMUs (see Figure 3). However, it seems relatively high; meaning low levels of efficiency and; subsequently opportunities to increase production through productivity gains. Surveyed farms should multiply their average production levels by 6.33 in order to reach the production frontier.
Studies conducted in Burkina-Faso (Ouédraogo and Kaboré, 1996) and elsewhere in the Sahel (Nyemeck, 2008) obtained similar conclusions, although with more varied efficiency scores, ranging from 16% to 74%.

Table 1. Descriptive statistics of Global Technical Inefficiency (GTIn) scores

Variables	Observations	Mean	St. D.	Min	Max
GTIn Scores biaised	321	4,89	4,36	1	44,24
GTIn Scores unbiaised	321	6,33	5,71	1,21	60,06
Biais	321	-1,44	1,38	-15,81	-0,20

Source : Authors` elaboration

To refine GTIn scores, factual analysis have been done using parameters such as: agricultural campaigns (years), cropping seasons, type of irrigated acreages, technics to collect water and types of production systems. The purpose is to disaggregate the global score into sub-scores related to each above mentioned parameters. Inefficiency sub-scores were compared using t-tests.
From this comparison, rainy season average inefficiency score is superior to dry season score, significantly. As crops are irrigated during the rainy season; irrigation is probably reducing the farm inefficiency level.
This result is intuitive given the fact that, irrigation practice requires more discipline in agricultural practices and production factors allocation. This is shown by the average inefficiency score of irrigated farms which is higher than the non-irrigated farms at 10% (see figure2). The decrease in irrigated crops efficiency would be linked to difficulties experienced by producers, following poor rainfall during the second agricultural campaign. In addition,

some crops planted during the dry season of the 3rd agricultural campaign (2010-2011) could not complete their production cycle. This inefficiency score may also be due to misallocation of water resources for crops; or could be interpreted as an inadequate consideration of climate risks into irrigated production schemes.

Farms with larger irrigated acreage seem significantly more inefficient than smaller irrigation acreage. This result is subject to questions and raises discussions on the overall performance of different types of size Indeed, as stated in the introduction, large irrigated acreages were regarded as entities with low economic and financial profitability; and where inefficiencies were observed in the majority of farms. In the context of Burkina Faso, renewed performance of large irrigated acreages could be induced by the combined effects of the return of specialized government offices in the process of framing and organizing producers; the support supplied to farmers organizations through subsidized agricultural inputs, etc.

In addition, the analysis shows that there is a negative correlation between the scores of GTIn and farm size (see Figure 3). From this, it follows that small farms have higher levels of inefficiency that large farms. Some explain the larger farms better efficiency with economies of scale.

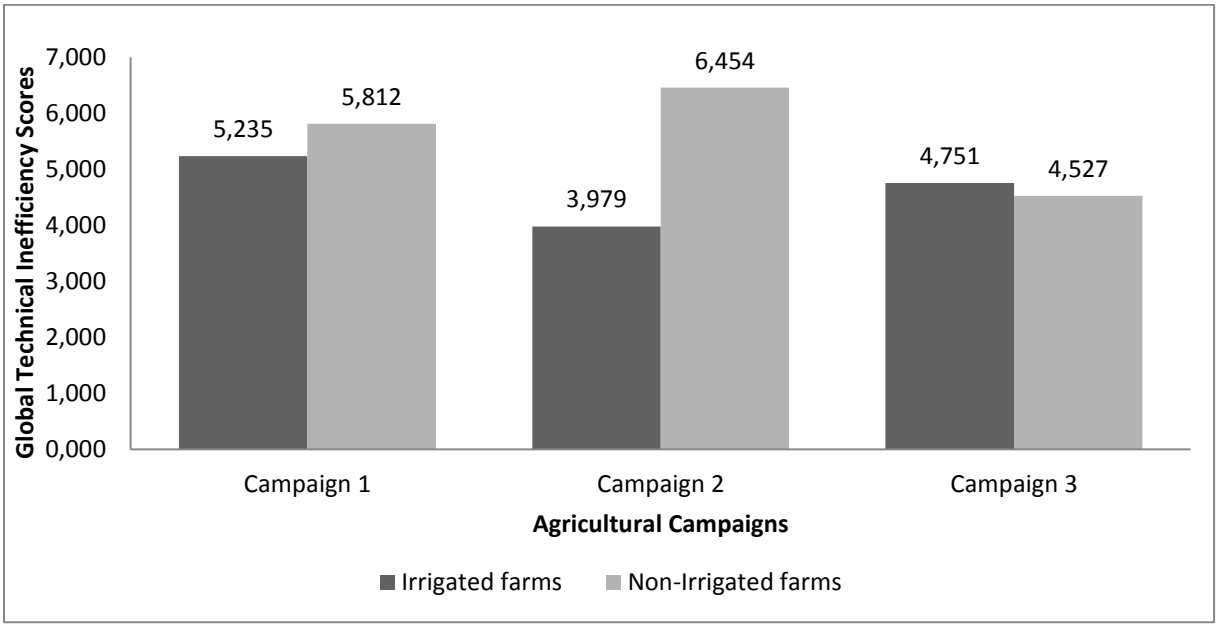

Figure 2: Global technical inefficiency over campaigns

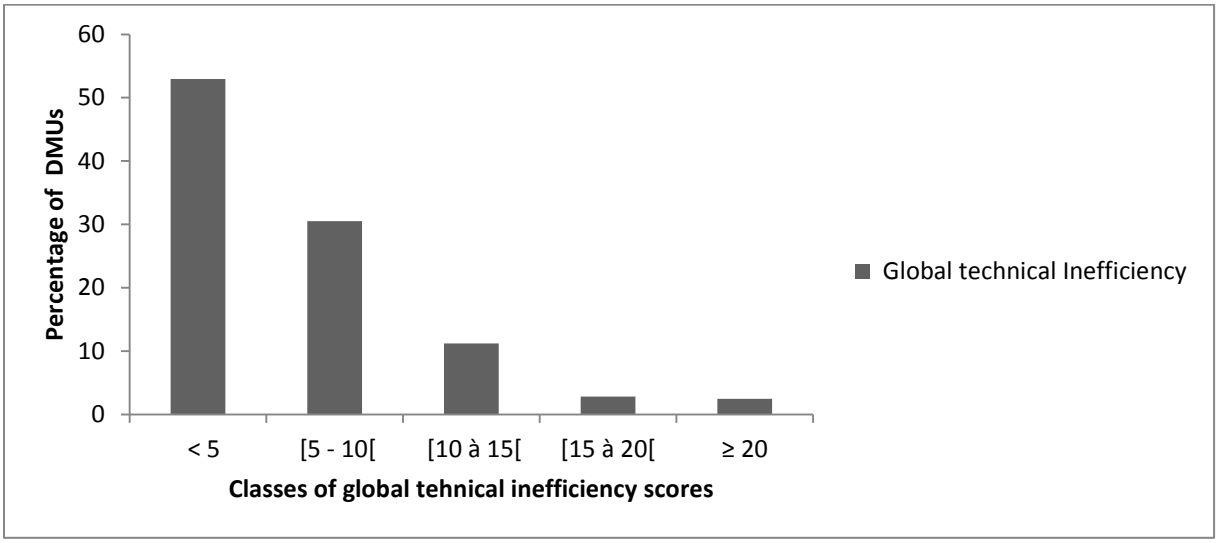

Figure 3 : Global technical inefficiency scores distribution

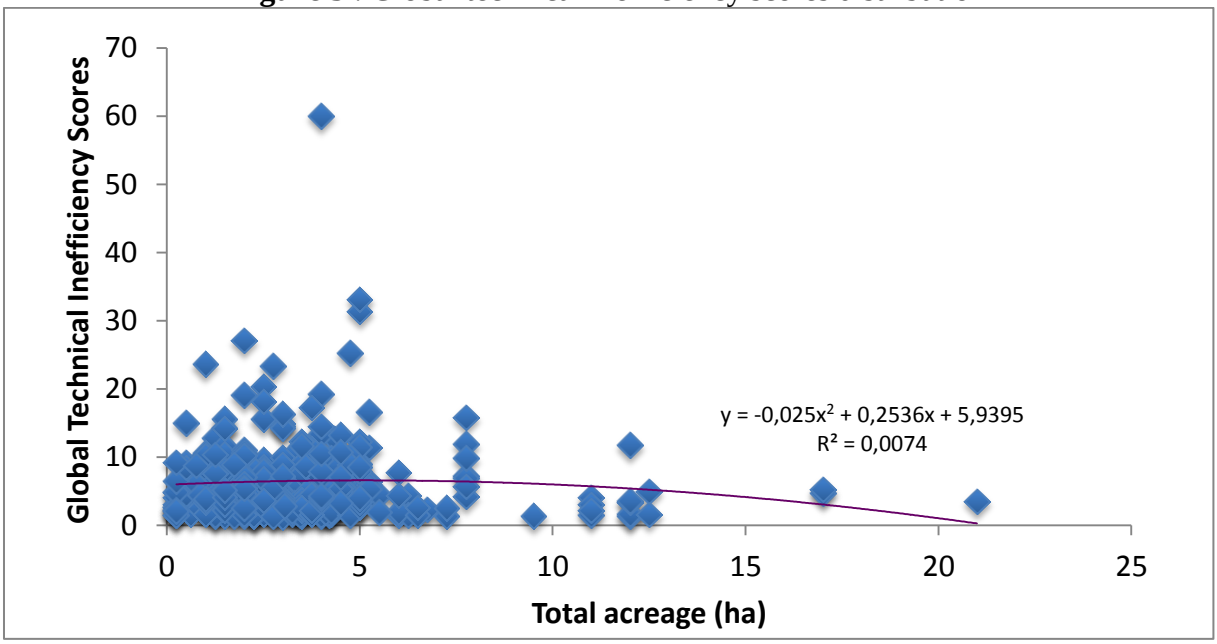

Figure 4: Distribution of the global technical inefficiency in terms of farm size

3.2. The Pure Technical Inefficiency and Scale Inefficiency

The global technical inefficiency is the result of pure technical inefficiency and scale inefficiency. The pure technical inefficiency refers to how inherently a DMU allocates its factors of production to achieve maximum production. The inefficiency of scale refers to the optimal size that allows a DMU to be on the production frontier. The analysis of these two parameters helps to refine and complete the development of the preceding paragraphs. As shown in Tables 5 and 6, it appears that the average scores of the pure technical inefficiency and the scale inefficiency for the observed farms are 5.06 (± 4.98) and 1.41 (± 0.86), respectively. In other words, these farms should improve the use of production factors in order to reduce the distance from the production frontier that is 5.06 points: therefore production could be quintupled. Also, optimal sizes allow these farms to increase their efficiency by 30%.

Figure 5 shows that globally, levels of pure technical inefficiency of farms decreased over time. This is probably due to effects of continuous technological learning, precisely for irrigation practice.

The increase of pure technical efficiency scores over time follows the same trend as the global technical inefficiency. While the scale efficiency decreased significantly from the 2nd to the 3rd cropping year. This increase in farms´ technical performance is expected in light of the new agricultural policies applied by Burkina-Faso government to support producers, since the 2008 food crisis.

Moreover, we note that throughout the period of observation, pure technical inefficiency of farms is significantly higher in the rainy season than in the dry season. In addition, farms with irrigated crops are significantly less inefficient than those practicing non-irrigated crops.

Statistically, it appears that, inefficiency scores average of farms on large irrigated schemes is significantly lower than those on small irrigated scheme. In addition, farms using pumps are significantly more efficient than those without pumps system. Irrigation without pumps is usually done through open canals downstream of dams. Such systems tend to waste more water than when it is pumped.

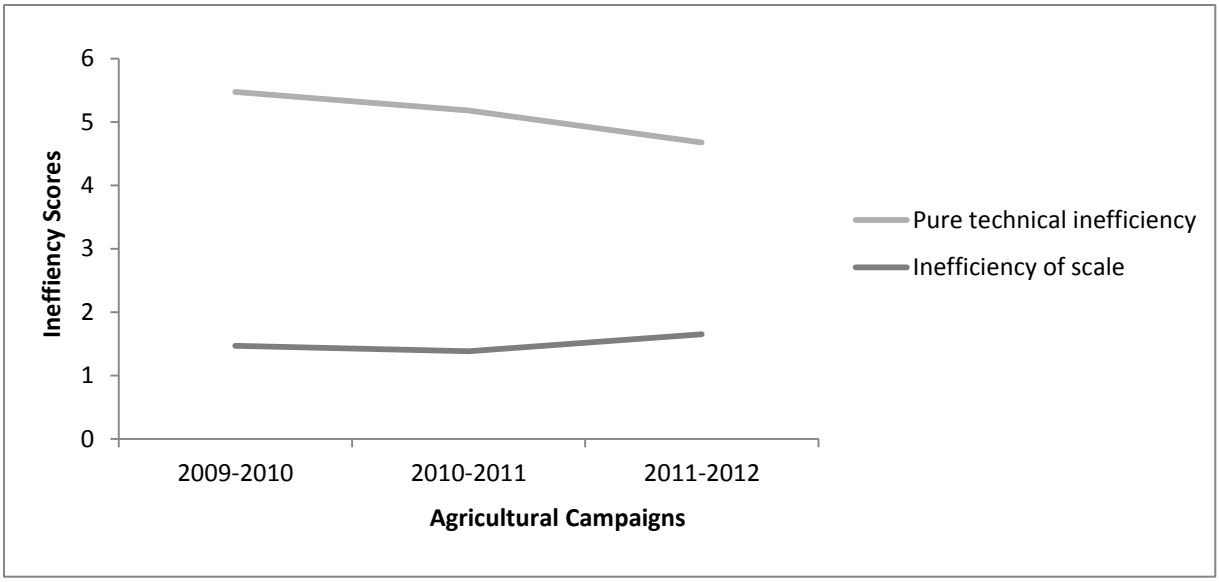

Figure 5: Pure technical inefficiency and scale inefficiency over campaigns

3.3. Factors explaining Technical Inefficiency

Table 2 . Results of technical inefficiency determinants OLS Model

Variable	CRS (TIn)	VRS (PTIn)	SE (SIn)
Gender	0,34 (0,90)	0,63 (0,84)	-0,33 (0,30)
Education level	0,16 (0,37)	0,11 (0,31)	0,07 (0,07)
Age	0,05 (0,03)	0,01 (0,03)	0,01 (0,01)
Irrigation (dummy)	-2,22 (0,88)*	-2,00 (0,79)**	0,06 (0,10)
Ratio paid /non-paid	-0,84 (0,46)*	-0,84 (0,43)*	-0,03 (0,09)
Number of Crops	-1,42 (0,35)***	-1,12 (0,31)***	-0,04 (0,06)
Household Size	0,09 (0,06)*	0,14 (0,04)***	-0,03 (0,01)***
Breeding Income	-0,00 (0,00)***	0,00 (0,00)***	0,00 (0,00)
Land Tenure	1,31 (1,27)	-2,34 (1,33)*	-0,80 (0,39)**
Constante	5,01 (2,53)*	2,51 (2,38)	2,42 (0,67)
R^2	0,08	0,10	0,12

* Significant at 10% level; ** Significant at the 5% level; *** Significant at the 1 %

The results of the regression estimation in Table 2 show that the socio-demographic variables such as gender, age and education level of farmers do not have a significant effect on technical efficiency level. This result is surprising because those are factors which should have improved the farms' efficiency (Gurgand, 1993; Phillips, 1994; Coelli and Fleming, 2004; Hasnah and al., 2004).

Irrigation practice has a positive impact on the global technical efficiency and pure technical efficiency of farms. I Irrigation might improve the water supply to crops; which improves the productivity through a better combination of farm inputs.

Considering the systemic approach that takes into account all crops grown by each farm, it turns out that crop diversification might be a key factor to significantly reduce the global technical inefficiency and the pure technical inefficiency.

Household size was revealed as a positive determinant of farms inefficiency. A large household size affects negatively the level of technical efficiency. But on the opposite, it reduces scale inefficiency.

The ratio of paid/non-paid worker is significant at 10%; and positively correlated with global technical efficiency and pure technical efficiency. This indicates that the use of hired workers, can significantly improve efficiency. From an economic point of view, this result indicates that it is probably profitable to contract hired labor

In addition, livestock production does not influence technical efficiency. This suggest that biophysical and monetary transfers between crops and livestock productions are not strong enough to impact significantly the farm production.

Following the partial assumption of variable returns to scale, the regression results show that land tenure plays a decisive role on pure technical efficiency. Confirming (Nyemeck, 2008) ; private land tenure promotes better production resources allocation.

In the current case, private land ownership would increase production by 2.34 (±1.33). However, land property rights reduce the inefficiency of scale. Therefore, land tenure policies should be set and easy to implement to permit to farmers to get better ownership rights.

Conclusion

The study analyzed irrigated farms technical efficiency in Burkina Faso, using panel data over a three-years period and a systemic approach; to estimate irrigated farm efficiency and to identify factors affecting these efficiency scores.

The results show that technical inefficiency of the studied farms is relatively low and has declined significantly over the three years of observations; unlike the inefficiency of scale. In addition, large irrigated schemes seem to be more efficient than the small ones. Also, the surveyed farms are more efficient during the dry season than during the rainy season. The practice of irrigation improves farms' efficiency.

Household head socio-demographic variables such as age, sex, and education level do not significantly influence efficiency. It is the same for livestock income. In return, the practice of irrigation, increased labor, crop diversification, household size and land tenure significantly determine the levels of efficiency of irrigated farms.

In terms of policy implication and strategies, one could recommend that :

- land reform underway in Burkina Faso should been accelerated to help securing farmers tenure in order to increase their efficiency ;
- irrigation is promoted with incentives towards crops diversification.

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FOREIGN TRADE OF FOOD PRODUCTS IN BOSNIA AND HERZEGOVINA

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Abstract

The foreign trade of food products in Bosnia and Herzegovina (B&H) has been investigated for recent period of three years (2012-2014). This research based on analysis of official data in B&H institution in the aim to established trend of trade of food, live animals, drinks and tobacco. In B&H, approximately the annually import of agricultural and food product is over 2 billion of Bosnian convertible marks (BAM) and export about 400 million BAM. Food production is a strategic interest of every country. The coverage of import by export was the highest (22.7%) in 2013 year, than (21.6%) in 2012 year and the lowest (18.4%) in 2014 year. During investigated period was established deficit of trade in B&H. According to potential of natural, social and economic resources in B&H, there is possibility for developing economy through increasing production, on the base of introduction modern technology and efficient use of capacity of domestic companies. Those enhancement will contribute to decrease import of food product. The key issues are defined of the food industry and the possibility of improving and enhancing foreign trade is suggested.

Key words: *food products, import, export, foreign trade of goods.*

Introduction

Food industry in B&H passes through the complex stages of recovery from the damage that occurred in the last decade of the 20th century, due to the war. In this period of political and economic destabilization of the Bosnia and Herzegovina's food industry, along with other economic sectors has done damage over 4 billion euros (IMF,1996). In the post-war period has started reconstruction of an economy that is in the transition phase included in the privatization program which is still ongoing. Also, the economy of Bosna and Herzegovina's develops on the specific connection but separately organized two separate economics i.e. economy of Republic of Srpska (RS) and economy of Federation of Bosnia and Herzegovina (FB&H). The add complexity is existing numerous economies of Canton within Federation of Bosnia and Herzegovina (FB&H). This organization influenced demographic changes, urbanization, local market and governance structures and processes, rural depopulation, industrial and agricultural modernization (FAO, 2014). The development altogether economy branch can help to identify and manage trade-offs and to build synergies which will allow more integrated and cost-effective planning, decision-making, implementing, monitoring and evaluating, (McCornick et al., 2008) program of agricultural production and food processing industry, to be more competitive.

During a few decades large area of arable land did not used for agricultural production and land did not protected from degradation, and land properties change with result in a decline in land quality. The data of estimates suggest that 5 - 6 million hectares of arable land worldwide are irreversibly lost each year as a result of soil erosion, salinization and other degradation processes. The land degradation affects agricultural productivity and productivity growth and food security which is influenced by economic, environmental and institutional factors. The important role of the farmer responses to land degradation and its potential

impacts on agriculture productivity and food security (Wiebe, 2003). The necessity is to improve the economic environment for farmers by developing market infrastructure, correcting distorted price incentives, and encouraging rural income growth and diversification (Radosavac, 2014).

In recent years, the food industry in B&H has a dynamic development, which includes the increase in production, especially agricultural products such as milk, certain species of fruit and vegetables. In this period of recovery, the food industry does not realize the possible volume of production because of the problems that accompany a particular congregation low level of utilization of the existing capacity of the manufacture. The main aspiration is to maximize the value of constructed capacities for processing raw potential, and thus to ensure conditions for increase of the unemployed population, an increase in production of agricultural products, increase exports while reducing imports and decrease in trade deficit with the maximum use of local resources (Radosavac and Pejanovic, 2012).

An Export of food products represents an important economic activity for the development of agriculture and food industry of B&H. In order to achieve better results in foreign trade with other countries in the region, it is necessary to explore the market and its products adapted to the requirements and needs of foreign consumers.

The aim of this work is to analyse of foreign trade of food products of B&H and the estimation of the possibilities of improving and expanding foreign trade, increasing competitiveness and exports, encouraging investment and entrepreneurship development.

Materials and methods

Paper is based on an extended literature review and data from different sources were used officially Agency of Statistics of B&H (ASB&H, 2015). Foreign Trade Chamber of B&H Chamber of Commerce of Republic of Srpska (MOFTER, 2015). Obtained data were used for analysis of import and export on the Standard International Trade Classification (SITC), for three years period 2011-2014. All obtained data were analyzed by using scientific descriptive methods, methods of analysis and synthesis attained and comparative analysis method.

Results and discussion

B&H has favorable conditions for development of agriculture and food industry. Natural resources in the RS and the Federation B&H, which are separate entities of B&H, represent the basic potential for the development of agriculture and food industry.

According to the structure of the products imported in all industries, from the food industry among the top 20 products imported are wheat, bread, biscuits, cakes, chocolate, various food products and beer. Most of the food products that are imported into B&H, can be produced in in the country, whose production has not been started.

Despite the favourable climatic conditions and production capacity of food industry, still there are numerous problems in this sector. The primary problems are that large areas of agricultural land not cultivated for many years and small abundance of domestic animals which can not satisfy even the minimum needs of the processing capacity and consumption.

Furthermore, key issues that arise in the food industry are: excessive import and unfair competition in the domestic market, low-cost finished products, the lack of state incentives for the production and export, the problem about funding etc., which leads to a reduction in productivity and competitiveness.

The countries with the greatest economic potential and political significance in region are Croatia and Serbia. Economic leader of the Western Balkans (WB) is the Croatia with the most competitive processing industry. There is great potential for trade growth in many countries of WB opposite the economic crisis that hit most countries. The recovery will

depend on economic developments in the EU, and the rapid recovery will influence the improvement of trade among the WB countries, attracting foreign investment, finding financial assistance from friendly and neighbouring countries.
During period of investigation in B&H, the volumes of foreign trade of food, drinks and tobacco was the highest (3.111 mil. BAM) in 2012 year and decline to 3.078 mil BAM in 2013 and the lowest 2986 mil.BAM. (ASB&H, 2014). Within total foreign trade in this period values of food, drinks and tobacco export variate from 568 mil. BAM (2013), 552 mil. BAM (2012) to 464 mil. BAM in 2014 year. The import of food, drinks and tobacco was five time higher than export in each year. The highest import 2558 mil. BAM was in 2012 , and the lowest 2509 mil. BAM in 2013. The import in 2014 was 2522 mil. BAM (fig. 1.). Bosnia and Herzegovina had deficit in foreign trade of food, beverages and tobacco in all three years of study. The largest deficit (-2.058 mil. BAM) was in 2014 and the lowest (-1941 mil. BAM) in 2013 year (Figure 1).

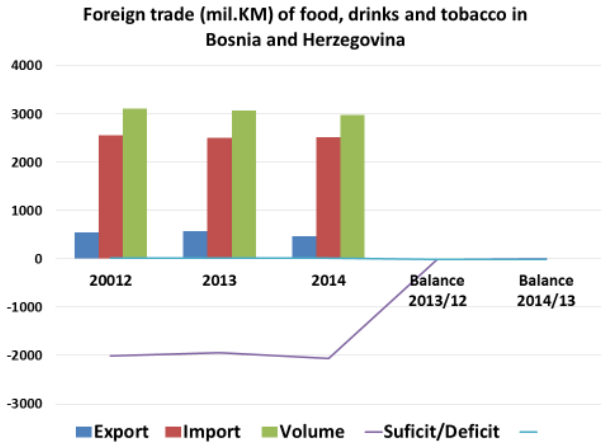

Figure 1. Foreign trade of food, drinks and tobacco in Bosnia and Herzegovina

Figure 2. Share of trade of food in total foreign trade of Bosnia and Herzegovina

Merchandise trade is based on the processing of primary products such as raw materials, semi-finished and finished products with a low added value. In commodity trade usually involved food products vegetables, fruit, confectionery, cereals behind the industrial product: petroleum products, gas, electricity articles of paper pulp, metals, aluminum profiles, textile products.
The share of food, drinks and tobacco in foreign trade variates in import and export in studied period. The value of export of food, drinks and tobacco have share of 7% in 2012 year, 6.8% in 2013 and 5.3% in 2014 year in compare to total foreign trade in B&H. The share of import of food, drinks and tobacco was 16.8% in 2012, 16.5% in 2013 and12% in 2014 year. For each year of trade, were registered deficit which was 27.1% in 2012, 28.6% in 2013 and 27.4% in 2014. The balance for the export varied from -0.2% for 2013/2012, and -1.5% for 2014/2013, while for the import the balance was -0.3% in 2013/12, and -0.9% for 2014/13. The balance for the suficit/deficit ratio was 1.5% for 2013/12 and -1.2% for 2014/13 (Figure 2).
The coverage of import by export was the highest 22.7% in 2013 year, than 21.6% in 2012 year and the lowest 18.4% in 2014 year (Figure 3).

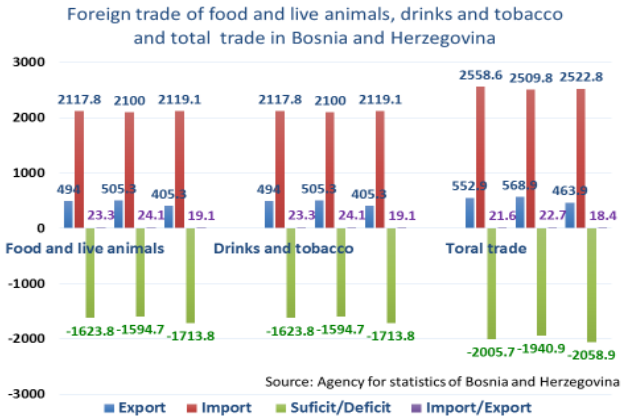

Figure 3. Coverage of import by export of food live drinks and tobacco in Bosnia and Herzegovina trade

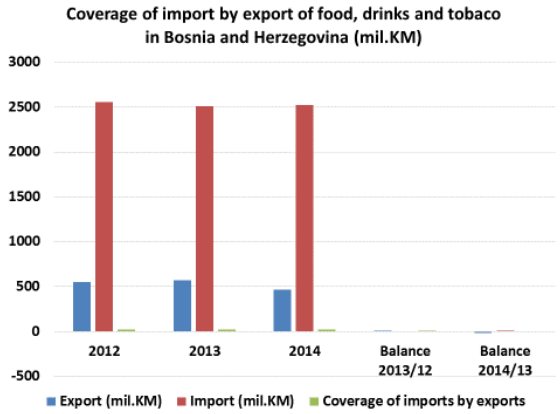

Figure 4. Comparison of foreign trade of food and animals with trade of drinks and tobacco in total in Bosnia Herzegovina

According to foreign trade data we found that in total export of food, drinks and tobacco, the share of export food and live animal was 494.0 mil. BAM in 2012 year, 505.3 mil. BAM in 2013 and 405.3 mil.BAM in 2014 year, while share of foreign trade of drinks and tobacco was 58.9 mil. BAM in 2012 year, 63.6 mil. BAM in 2013 and 58.6 mil.BAM in 2014 year (Figure 4).

The share of food and live animals in total in import was 2117,8 mil.BAM wit deficit -1623.8mil.BAM in 2012 year, while in 2013 year 2100 mil.BAM with deficit -1594.7mil.BAM and in 2014 year 2119.1 mil.BAM with the highest deficit -1713.8 mil.BAM. For the drinks and tobacco the share of import was 440.8mil.BAM in 2012 year, 409.8 mil.BAM in 2013 year and 403.7 mil.BAM in 2014 year. Deficit of trade of drinks and tobacco was -381.9 mil.BAM in 2012 year, -346.2 mil.BAM in 2013 year, -345.1 mil.BAM in 2014 year. (ISFB&H, 2014).

The coverage of import by export in 2014 year was 19.1% for food and live animals, and 14.5% for drinks and tobacco.

Due to insufficient domestic capacity utilization in B&H, there is a very large import of all types of food products, especially products of lower quality than domestic products. The largest import of food products originating from neighbouring countries Croatia and Serbia, with which B&H and RS have concluded a free trade agreement, with 0% tariff rate for imports.

Conclusion

In this study were established no-balance of export and import of food, drinks and tobacco in B&H for the three years period 2012-2014. Although there are high potential for food production in B&H, the four fold is higher import than export of food, drinks and tobacco. The coverage import by export variates and was 21.6% in 2012 year, tightly higher 22.7% in 2013 and decreased in 2014 year (18.4%). The high VAT rate of 17%, in comparison to other countries where the VAT ranges from 5-8% what contribute to a significant reduction of competition and import of low quality products with low price what and automatically created unfair competition.

The value and trend of foreign trade of food, drinks and tobacco of B&H have negative influence on domestic production and agricultural producers. In the future is necessary resolve key problems between production and consumption, domestic savings and investment levels,

as well as optimize of utilization of country's available resources: water, land, good geographical location, neighborhood to the EU market, workers, staff.

Bosnia and Herzegovina can make progress of production and trade of food products on the base of developing new program of investment, modernization of processing capacity, innovative technology of production in cooperation with the Western Balkan Countries and developed Countries in EU and all over the World. The high potential is possible realize by increasing export to Croatia. The Government measure and support of the Ministry of Agriculture, Water Management and Forestry have important role for increasing export food product and protection domestic production from unfair foreign competition.

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THE EFFECT OF GLOBAL G.A.P. ON THE FINANCIAL RESULTS OF AGRICULTURAL HOLDINGS IN BOSNIA HERZEGOVINA

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Abstract

Aspects of the protection of natural resources, environmental management, workplace safety, animal health and well-being, food safety, healthcare and the general principles promoted by standardization i.e. the norms and demands of GLOBAL G.A.P. are rarely applied in most Bosnian and Herzegovian agricultural holdings. The markets of most transition economies, including Bosnia and Herzegovina, are flooded with goods of dubious quality in terms of biological value and hygiene. The once achieved quality of the product that aims to be constantly competitive in the foreign market requires much more than just tradition and a well-designed label. In this study a comparison was made between the agricultural holdings which apply the standard and those which base their production on the conventional “ad hoc” system. The effects of the application of standards on the overall business of agricultural holdings that produce fruits and vegetables were examined. Ultimately, as proof, we will see a reduction in production costs and an improvement in overall financial results of the agricultural holdings that were being examined.

Key words: *quality, standardisation, GlobalG.A.P., production costs, financial results.*

Introduction

Quality concern refers to the procurement of raw materials, finished product production, storage, sale, distribution, waste disposal, consumer information and other relevant characteristics, as seen through the prism of cost reduction of agricultural products. Nowadays, the product certification of production according to the GLOBALG.A.P. norms appears as one of the key recommendations of retail chains directed towards the agricultural product suppliers.

The aim of this study is to determine the importance of the implementation of standards and their impact on the overall costs of production at the selected agricultural holdings which are subcontractors of the agri-businesses (the “Voćar Piramida” company from Visoko; the agricultural cooperative “Gračanka”, and “PMV-VIP” from Gradačac). The analysis of production costs proved the improvements in business and the competitiveness in the domestic market, as well as the EU market, of those agricultural holdings which implement the GLOBALG.A.P. standards in relation to those agricultural holdings which base their food production on the conventional “ad hoc” system.

The implementation of the above mentioned standard has proven that the realisation of the achieved market value of production is better and securer, and it leads to the reduction of the overall costs of production, thus increasing productivity. In addition to cost reduction (of all inputs required for production, with an emphasis on the chemical plant protection products and mineral fertilizers), non-measurable positive effects and merits for the broader community through environmental improvement also occur. It is necessary to prove that the application of the GLOBALG.A.P. standards increases the solvency and efficiency of agricultural holdings, as well as its competitiveness in the domestic market and the EU market, which ultimately leads to better financial results in relation to the holdings which base their production on the conventional means of production.

Materials and methods

On the way to joining the European agricultural sector, agricultural holdings in Bosnia and Herzegovina have to undergo fundamental changes i.e. the tasks of conducting organizational and economic measures in order to comply with the EU standards. The situation in the field of records and business analysis of agricultural holdings is particularly complex. This kind of situation puts the owners at a disadvantage because they are unable to quantify investment or achieved results. The most important data necessary to make such decisions relates to costs of production, as well as the results achieved during the year. This data is used to calculate the business performance of agricultural holdings. Such a record system should serve the agricultural holding for the purpose of adjusting its own production. The big problem for achieving large scale production is the fragmentation of the areas of agricultural holdings. The solution to this problem could be found in association of the agricultural producers into cooperatives.

Modern managerial functions of product quality management system using the GLOBALG.A.P. system and other standards increase the success of the business through reduction of the overall costs. The comparison was made between the actual financial results from the annual financial statements, taking into account actions that are taken in a given year in terms improving the product quality and control of business processes. The research was conducted in three agri-businesses : Voćar Piramida” Visoko, ZZ „Gračanka“ Gračanica i „PMG-VIP“ Gradačac, which trade in both purchased and self-grown produce in the agrarian sector. The cooperatives organise a significant volume of production with an impressive number of subcontractors.

Table 1. The structure of the areas of production of agricultural holdings in 2014

Agricultiral cooperative	Arable land/ha	Total area in production/ha	Fruits	Vegetables	Other - grains
Z Z „Gračanka“ Gračanica	118	109	41	23	45
„Voćar Piramida“ Visoko	64	38	25	4	9
„PMG-VIP“ Gradačac	52	44	16	9	19
T O T A L	234	191	82	36	73

These cooperatives successfully completed the certification process of GLOBALG.AP standards three years ago, which contributed to the producers, in north-eastern Bosnia and Herzegovina, placing large amounts of fresh fruits and vegetables on the demanding European Union market.

Regardless of the fact that the focus of the research included only three legal agri-businesses, we can claim with certainty that these rationales could be roughly applied to the total area of Bosnia and Herzegovina.

All household members are capable of working on any basis.

In order to facilitate the application and understanding of the GG, the level of education is very important. 60% of subjects have secondary and tertiary education which enables the application of GG.

Number of persons in the household shows that these are agricultural holdings that have good prospects of development.

In the majority of subjects (70%), the quality of agricultural products is above average i.e. falls into the high quality category.

The problems which these holdings face in their businesses are diverse: low prices, poor equipment and machinery, poor solvency etc.

The majority of agricultural holdings owners stated that they owned only one part of the equipment and even those parts were outdated; the smaller percentage of the holdings are satisfied with their machinery and tools.

Subjects are not satisfied with the amount of training concerning the modernization of production which they received from the agricultural entities. They would like to participate in more seminars and training.

The surface area of the holdings is very important in terms of reducing the costs of utilization of tools and machinery, the largest number of subjects is in the 'over 3 hectares' group

All surveyed agricultural holdings are of mixed type.

As regards to the storage space, all surveyed holdings gave a satisfactory solution.

The value chain requires a radical change that would improve the position of agricultural producers.

The majority (44%) have stated that the effects of the GLOBALG.A.P. certification are very positive.

The research used the subject breakdown method: financial results and costs, as well as the calculation of the cost price of a product unit before and after the introduction of GLOBALG.A.P.

Results and discussion

The analysis was focused on five kinds of fruit: plums, apples, pears, raspberries and blueberries, and three kinds of vegetables: tomatoes, peppers and cucumbers.

Table 2. The distribution of values among the stakeholders in the value chain of fruit

Serial No.	**Name of the fruit variety**	**Price in BAM[16]**		
		Agricultural holding	**Agricultural cooperative**	**Market**
1.	*Plum*	0.80	1.00	1.60
2.	*Apple*	0.60	0.85	1.10
3.	*Pear*	1.00	1.25	1.60
4.	*Raspberry*	3.00	4.20	6.80
5.	*Blueberry*	3.50	4.80	7.50

In the analysis of the value chain of the individual fruit crops it is evident that the distribution is mainly at the detriment of the producers, the buyers (ZZ „Gračanka"; „Voćar Piramida" Visoko; „PMG-VIP" Gradačac) have a significantly higher value which is a result of storage and possible processing. If the buyers (the analysed legal entities) and exporters are relative in the aspect of trade, than the profit of all the listed fruit varieties is 100% higher compared to the price of the products purchased from the agricultural holdings.

[16] BAM-Bosnian Covertible mark is Bosnian currency

Table 3. The distribution of values among the stakeholders in the value chain of vegetables

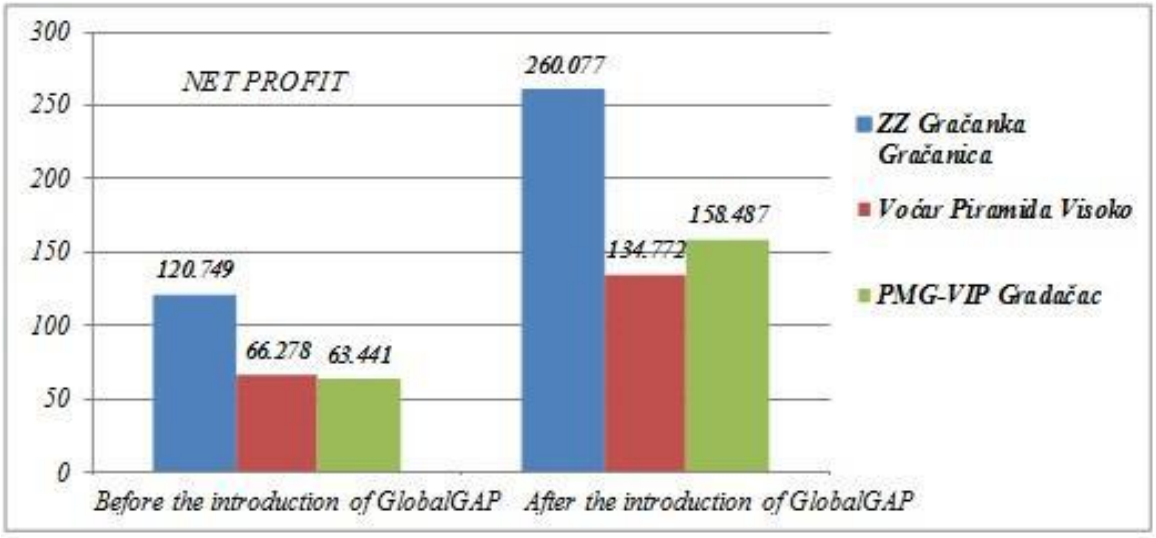
Serial No.	**Name of the vegetable variety**	**Price in BAM**		
		Agricultural holding	**Agricultural cooperative**	**Market**
1.	*Tomato*	0.60	0.80	1.00
2.	*Pepper*	1.80	2.20	2.80
3.	*Cucumber*	0.30	0.40	0.60

The difference in relations in the value chain of vegetable crops is not significant. True, there are significant financial investments from buyers, including manpower for the reception and processing, use of storage and cooling capacities, the use of equipment, etc. All these costs burden the product with 25 - 30% on average.
Analysis of the structure of financial results was done before the introduction of GLOBALG.A.P (2011-2012) and after the introduction of GLOBALG.A.P. (2013-2014)

Table 4. The structure and disposition of total revenues for 2011-2012 before the introduction of GLOBALG.A.P. and for 2013-2014, after the introduction of GLOBALG.A.P.

Economic indicators	**Before the introduction of GLOBALG.A.P.**			**After the introduction of GLOBALG.A.P.**		
	Z. Z. „Gračanka“ Gračanica	**„Voćar Piramida“ Visoko**	**„PMG-VIP“ Gradačac**	**Z. Z. „Gračanka“ Gračanica**	**„Voćar Piramida“ Visoko**	**„PMG-VIP“ Gradačac**
2011-2012				***2013-2014***		
1.Operating revenue	*3.364.200*	*1.645.000*	*1.882.350*	***4.205.663***	***2.056.443***	***2.185.332***
2.Financial revenue	*226.450*	*121.135*	*155.680*	***241.200***	***287.050***	***291.330***
3.Other revenue	*364.560*	*222.870*	*187.300*	***211.450***	***121.338***	***312.440***
4.Total revenue (1 to 3)	***3.955.210***	***1.989.005***	***2.225.330***	***4.658.313***	***2.464.831***	***2.789.102***
5.Operating expenses	*3.602.235*	*1.611.223*	*1.968.970*	***3.960.446***	***2.112.205***	***2.403.776***
6.Financial expenses	*206.400*	*335.450*	*175.900*	***380.500***	***188.660***	***201.330***
7.Other expenses	*12.410*	*7.680*	*9.970*	***28.400***	***14.220***	***7.900***
8.Total expenses (5 to 7)	*3.821045*	*1.915.353*	*2.154.840*	***4.369.346***	***2.315.085***	***2.613.006***
9.Gross profit (4-8)	***134.165***	***73.652***	***70.490***	***288.967***	***149.746***	***176.096***
10. Loss (8-4)	-	-	-	-	-	-
11. Income tax expense	***13.416***	***7.365***	***7.049***	***28.890***	***14.974***	***17.609***

12. Net profit (9-11)	***120.749***	***66.287***	***63.441***	***260.077***	***134.772***	***158.487***
13. Net loss	-	-	-	-	-	-



Graph 1. Financial indicators of agricultural legal subjects with and without GLOBALG.A.P.

Through the analysis of the individual business performance the average revenue of certain types of products for the international market has increased by 32%, whilst the turnover and the price in the domestic market have increased by 22%. In relation to the total of other revenues with the same production volume and the same product structure, the gain is 100% higher in the period of the GLOBALG.A.P certificate. The reason for this is the increase in the prices of final products.

Graph 2: *A comparison of profits in BAM with and without GLOBALG.A.P. for agricultural subjects*

Based on the results obtained by comparative analysis, it can be concluded that the introduction of GLOBALG.A.P. into the business philosophy of all the agricultural subjects was profitable (which means that the price of the funding sources was covered and the achieved accumulation is significantly higher in the period of GLOBALG.A.P. performance compared to the period without the certification), and the operating revenue was increased in both domestic and foreign markets.

Conclusion

The level of knowledge of all participants in the value chain of the processes is very low and there is no vertical and horizontal synchronisation. The agricultural subjects (who were the focus of the research) that deal with the purchase and processing of fruits and vegetables are lacking initiative for a higher degree of the processing of the product, in order to achieve greater added value for the product for all participants in the value chain. The producer gets the lowest value of all participants in the value chain (two or three times less compared to the final market price). Standardization is inevitable in agriculture and the food industry. The gap in the modernization of production could be bridged by the use of traditional knowledge and skills and their integration into the standards. The fact is that the certified product is export-oriented and highly profitable. The analysis of the individual business performance determined the increase of the average revenue of certain types of products for the international market by 32%, whilst the turnover and the price in the domestic market have increased by 22%. In relation to the total of other revenues with the same production volume and the same product structure, the gain is 100% higher in the period of the GLOBALG.A.P certificate. The reason for this is the increase in the prices of final products.

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THE ROLE OF SUSTAINABLE REPORTING TO PERFORMANCE MANAGEMENT IN THE RETAIL FOOD

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Abstract

Lately, the greater the role and importance of sustainable reporting as an instrument of performance management in all economic sectors, including food retailers. The sustainable development concept is increasingly used in the food sector because of its importance. It is applied across the entire food value chain. The characteristics of integrated sustainable food system are: environment - energy and climate changes, water use, biodiversities, land use, soil health; sociality - development, animal welfare, social equity, and economics - food safety and nutrition. What is predominantly used in the retail sale of food, (which is member of the value chain), is the sustainable development. In that context, considerable importance in modern retail is given to the effective protection from loss, as well as the rational food waste treatment, including packaging. A special sustainability indicators system is developed in the food retail. It enables more effective control of the concept applying effects of sustainable development in food retailing. All this contributes to creating sustainable value in food retail. This is particularly important for food retailers during the economic crisis.

(**JEL Classification:** D40 L81 M41)
Keywords: *sustainable value, food value chain, food waste treatment, organic food value chain, sustainable reporting*

Introduction

Lately, most of the attention is paid to the sustainable development concept application and its effects. According to the United Nations, sustainable development is defined "*as development that will meet the needs of the present without compromising the ability of future generations to satisfy their own needs*" (Our Common Future, World Commission on Environment and Development - WCED, 1987). It integrates three dimensions: environmental, social and economic, and requires interdisciplinary approach to the analysis of sustainable development. The primary elements of sustainable development (which is usually displayed in the form of a spider web) are: natural resources, food, water and land; policy and control, economic development, technology, social issues related to the employment of the poor, human rights and democratic participation; health; population, education, environment, lifestyle, values and power. Sustainable development is therefore a key element of the environmental policy (Nilsson, 2012).

Significant attention, both in theory and in practice, is dedicated to the development of an appropriate system of sustainable reporting (Golini, 2011; Lukic, 2011; Lukic, 2012; Lukic, 2013; Smith, 2008; Soosay, 2012). A research, (for example, a Fortune 500 company), determined that sustainable development reporting is done largely through the websites in all economic sectors, including retail. Reporting on the development of green practices via the website is much higher in the retail and service than in manufacturing. There are a number of initiatives for sustainable development in the retail business: The global reporting initiative (Global Reporting Initiative), Stock Market Indices of sustainable reporting (Johannesburg Stock Exchange - JSE), Project on disclosure of carbon emissions, water consumption disclosure Project, Dow Jones sustainable Index, and the Global Compact of the United

Nations. A special international standard of organization is developed (ISO 2600 for Social Responsibility), which allows (to all firms, but also to the retailers) improving the general conditions of work, and has a positive impact on sustainable development. Its main characteristics are: state organization, human rights, labour practices, the environment, fair operating practices, customer's issues, community involvement and development (Bekele, 2012).Global retailers define their criteria for sustainable business. The ultimate effect of the development and implementation of sustainable development are to improve the performance of retail companies measured by sustainable value. In this paper, the term sustainable value to include the effects of applying the concept of sustainable development in retail foods. The main objective of this research is to analyze the most important issues for sustainable value in food retail. The main contribution of this study is that it suggests ways for the scaling up of sustainable value in food retail. Likewise, indicating a need given the importance of the introduction of a special sustainable reporting in modern retail food companies.

Material and Methods

Research methodology of given hypothesis is based on the study of literature, norms, and comparative analysis of empirical data by country, types of shops and food categories. Analyzed original empirical data were collected from the literature, and corporate annual reports. Significant attention, both in theory and in practice, is dedicated to the development of an appropriate system of sustainable reporting. A research, (for example, a Fortune 500 company), determined that sustainable development reporting is done largely through the websites in all economic sectors, including retail. Regarding it, there are certain differences in individual sectors of the economy. Reporting on the development of green practices via the website is much higher in the retail and service than in manufacturing. Given the fact that the unequal application of sustainable reporting, rather a low level, in the retail sector of food, limited the comparability of sustainable data globally.

Results and Discussion

Recently, considerable attention, both in theory and in practice, is dedicated to the sustainable development of retail, especially consumer goods (food). It is quite understandable taking into account that *sector of retail consumer goods is very important* in the world by the revenue generated by sales and number of employees. They are, by their geographical origin, mainly from the United States, Germany and France. The essential features of global retailers of consumer goods share in total revenues of the top 250 retailers in 2013 were: % retail revenue from foreign operations 23.2%, average countries 4.9% and % single country operators 41.7% (Source: Deloitte - Global Power of Retailing Report 2015).The food industry is the first in the European Union by its revenue, with more than 8 million employees (Manzini, 2013). France, Germany, and the United Kingdom are the countries with significant retail trade in food . In addition to the development of private brands, the essential characteristics of the modern food retail is growing sales of organic food. Having considered its nature, food *value chain* is specific compared to other products. The typical life cycle of food value chains is the production, processing, distribution, retail, consumption and waste treatment. Integrated value chain of food and supply chain has been incorporating increasingly sustainable dimensions in recent years. The so-called sustainable food value chain is being developed. Sustainable supply chain is different for each type of food. *The value chain is specific to each category of organic food* (Table 1, Figure 1).

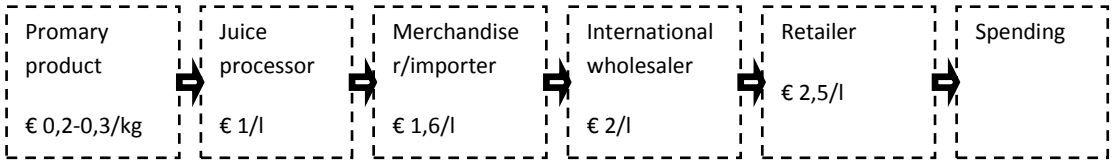

Figure 1. The value chain of organic production in Serbia - the case of apple juice

Source: Miskovic et al (2013).

Table 1. U.S. organic food sales by category, 2005-14E

($ billion)

	Fruit and vegetables	Dairy	Beverages	Packaged/prepared foods	Breads and grains	Snack foods	Meat, fish, poultry	Condiments
2005	5.369 (40.3%)	2.14 (16.1%)	1.657 (12.4%)	1.627 (12.2%)	1.36 (10.2%)	0.561 (4.2%)	0.256 (1.9%)	0.341 (2.6%)
2006	6.068 (39.0%)	2.579 (16.6%)	1.934 (12.4%)	1.887 (12.1%)	1.651 (10.6%)	0.68 (4.4%)	0.345 (2.2%)	0.417 (2.7%)
2007	6.932 (38.0%)	3.081 (16.9%)	2.302 (12.6%)	2.164 (11.8%)	1.949 (10.7%)	0.84 (4.6%)	0.476 (2.6%)	0.522 (2.9%)
2008	7.799 (38.0%)	3.406 (16.6%)	2.599 (12.7%)	2.396 (11.7%)	2.133 (10.4%)	0.949 (4.6%)	0.606 (3.0%)	0.636 (3.1%)
2009	8.658 (40.1%)	3.373 (15.6%)	2.587 (12.0%)	2.498 (11.6%)	2.21 (10.2%)	0.972 (4.5%)	0.618 (2.9%)	0.675 (3.1%)
2010	9.689 (41.4%)	3.681 (15.7%)	2.708 (11.6%)	2.574 (11.0%)	2.328 (9.9%)	1.06 (4.5%)	0.644 (2.8%)	0.724 (3.1%)
2011	10.844 (42.2%)	4.028 (15.7%)	2.921 (11.4%)	2.768 (10.8%)	2.48 (9.6%)	1.163 (4.5%)	0.724 (2.8%)	0.783 (3.0%)
2012E	12.145 (42.7%)	4.308 (15.2%)	3.203 (11.3%)	3.02 (10.6%)	2.671 (9.4%)	1.331 (4.7%)	0.846 (3.0%)	0.888 (3.1%)
2013E	13.55 (43.1%)	4.663 (14.8%)	3.506 (11.1%)	3.325 (10.6%)	2.896 (9.2%)	1.517 (4.8%)	0.986 (3.1%)	1.001 (3.2%)
2014E	15.06 (43.3%)	5.071 (14.6%)	3.839 (11.0%)	3.683 (10.6%)	3.157 (9.1%)	1.724 (5.0%)	1.141 (3.3%)	1.122 (3.2%)

Note: E=estimate.
Source: USDA, Economic Research Service using data from Nutrition Business Journal.

Specific *indicators for measuring sustainable value in the food sector* are developed. Environmental impact indicators are: biodiversities, climate / energy, waste, toxicity and water. Indicators of social impact: the right of workers and social health care. Indicators of economic impact are: profitability of farms, livelihoods – wage, and the elasticity of the value chain. A special system of sustainable indicators of food supply chain is developed. Table 2 shows sustainable indicators for food supply chain in the United Kingdom. Global retailers develop their indicators of sustainable business (Maria A.O. Dos, 2013).

Table 2. Sustainable indicators for food supply chain in the United Kingdom

Fazes	Economy	Social	Environment
Agriculture	Output Productivity Profitability Market Import vs. Domestic Distribution of import	Employees Average salary Dangerous matter Health and care Fair trade	Environmental reporting Animal welfare Energy usage Water usage Packing usage Waste Harvest loss Diversity of breeds
Processing	Output Productivity Profitability Market Import vs. Domestic Distribution of import	Employees Average salary Dangerous matter Health and care Fair trade	Environmental reporting Energy usage Water usage Packing usage Waste Food loss
Distribution	The growth of output Work productivity Profitability Market	Employees Average salary Dangerous matter Health and care Fair trade	Environmental reporting Energy usage Water usage Packing usage Waste Food loss
Spending	Food access	Fresh vs. processed food Fair trade	Food loss Waste

Source: Yakoveleva, (2005)

Global retail company *Wal-Mart* gives special attention to the issue of sustainable development and sustainable cost effective management in the function of optimizing operational and financial performance (i.e., creation of additional value). It has developed an adequate network of sustainable value, which is displayed in Figure 2.

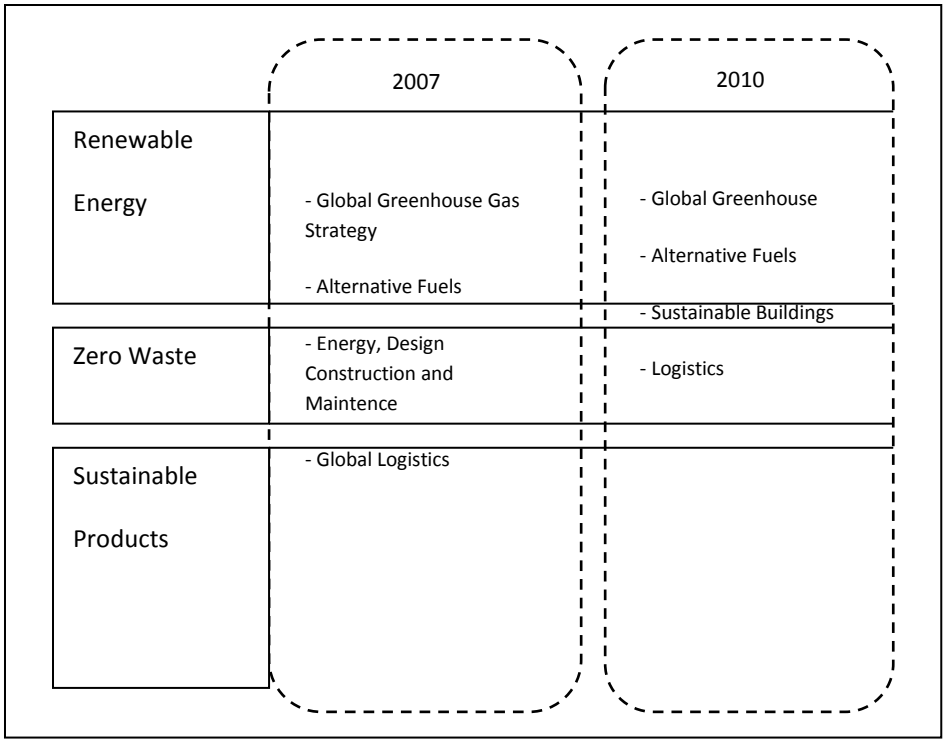

Figure 2. Wal-Mart's Sustainable Value Networks

Source: Lukic, (2013)

Wal-Mart achieved significant cost savings from reducing carbon dioxide emissions, ensuring efficient transport vehicles (in terms of fuel consumption) and making special packaging. Since 2005 company Wal-Mart regularly publishes (on the website) annual report on sustainable development and its sustainability objectives are: 1) the use of 100% renewable energy, 2) providing zero waste, and 3) the sale of products that are sustainable to environment and people (Lukic, 2013). These goals are achieved through reduction of carbon dioxide emissions, increase of energy efficiency, reduction of transportation costs, waste reduction and recycling, packaging and so on.Walmart plans to build such stores with 25-30% greater energy efficiency, which will produce up to 30% less carbon dioxide emissions. Also, the plan is to carry out the reduction of carbon dioxide emissions by 20% in existing stores around the world (Lukic, 2013). It will be beneficial to reduction of overall costs and increase of profits at the time, with the maximum satisfaction of the needs and desires of consumers.
Sustainability is integral part of the new business model - global strategies of operations of *Delhaize Group*. Companies Delhaize Group significant attention to the development and application of the concept of sustainable development and creating sustainable value in their business. The effects are visual in terms of energetic efficiency, reduction of water consumption, waste treatment, safety of food and health, environmental protection, human resource management, corporative social responsibility (Table 3). It has a positive impact on its overall business and financial performance.

Table 3. Zero Waste in Delhaize Group

	2020 Zero Waste Goals	2014 Progress
Recycling **(*)**	80% of waste will be recycled	58% of waste recycled
Food Donations **(*)**	100% of stores and warehouses will have Food Donation Programs in place to maximize the amount of surplus food that is donated to food recovery charities	We have a long history of contributing unsold food to food banks, and are now determining how to maximize those donations. New reporting against this target will start in 2015. 27 000 tonnes of food donated to food banks
Greenhouse Gas Emissions	Achieve 20% reduction in CO_2 equivalent emissions per m² of sales area (against 2008 baseline)	0.599 tonnes CO_2 e / m² 8% reduction since 2008 14% reduction since 2008 for operating companies excluding Delhaize Serbia
Refrigerants **(+)**	Refrigerants will be 80% ozone-friendly and will have an average global warming potential (GWP) of 2230 or lower	55% of refrigerants are ozone-friendly, based on total refrigerants in our systems 2362 average GWP, based on total refrigerants in our systems

(*) = new goal in 2014 **(+)** = revised goal in 2014
Source: 2014 Annual Report – Delhaize Group

Conclusion

The main objective of this research is to analyze the most important issues for sustainable value in food retail.. The main contribution of this study is that it suggests ways for the scaling up of sustainable value in food retail. Likewise, indicating a need given the importance of the introduction of a special sustainable reporting in modern retail food companies. Given the fact that the unequal application of sustainable reporting, rather a low level, in the retail sector of food, limited the comparability of sustainable data globally.

Recently, considerable attention has been paid, both in theory and in practice, to sustainable development in food retail. It is quite understandable when one takes into account the fact that the food retail sector is one of the most important in the world by the revenue from sales and number of employees. In this context, sustainable reporting is increasingly important as a significant instrument for performance management of retail food chains.

Typical lifecycle of food value chains is production, processing, distribution, retail, consumption and waste treatment. Integrated food values chain or food supply chain has been increasingly incorporating sustainable dimension in recent years. A sustainable food value chain is developed. As a part of that, great attention is paid to effective management of losses and food waste, including packaging. Specific indicators have been developed to control the effects of sustainable development in the food retail market. The effects of this are improved performances of food retailers. In addition, there are significant benefits in terms of health and safety and for the buyers of food, as well as environmental protection.

Lately, the development of sustainable organic food has an increasing importance in both global level and the individual countries. The value chain of organic food production is specific. In modern retail, the share of organic food in total sales has increased. This tendency will continue in the future.

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EVALUATION OF ECONOMIC EFFICIENCY OF SOLID BIOMASS PLANT CONSTRUCTION

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Abstract

Bosnia and Herzegovina (BaH) is exporter of electricity, which is mainly produced from water potential and coal. Significant reserves for production of additional quantities of electricity are found in agriculture and forest waste. One of renewable energy sources is crop residues biomass after harvesting. The subject of research is possibility of utilization of grain residues biomass for electricity production. The research objective is to evaluate economic efficiency of investment in construction of biomass plant of installed capacity of 300 kW. The analysis made for a municipality in the northern Bosnia and Herzegovina (Pelagicevo) confirmed that 3,000 ha of arable land under small grains and maize can ensure 12 thousands tones of biomass annually, out of which 30% can be extracted for electricity production. In the biomass power plant (active power of 250 kW) out of 2,100 tons of biomass, 1.5 million kWh of electricity can be produced. The analysis of economic efficiency of investment of 967,504 KM (KM=convertible mark) into construction of biomass power plant, conducted by dynamic methods of investment evaluation confirmed that such investment is in a long term efficient since its twenty-year net present value, by using a discount rate of 8%, is positive (NPV=90,213 KM), the invested money pay back in 18.08 years and internal rate of return (IRR=9.19%) is higher than the cost of capital from which the project is financed. In addition to individual financial benefits for investor, social benefits of these investments are also important (pollution reduction, production of "green energy", increase in employment, additional income for farmers, etc.).

Keywords: *crop residues, biomass, biomass plant, project appraisal, economic efficiency.*

Introduction

Biomass is a renewable resource of biological origin. Biomass is becoming increasingly interesting worldwide. It is the most commonly used directly as energy for heating, cooking and water heating, but it can also be used to produce electricity and heat, and recently it has been increasingly used for biofuel production. As defined in the Rulebook on promotion of electricity generation from renewable sources and efficient cogeneration (Offical Gazzete of the Republic of Srpska, 2013) biomass means the biodegradable fraction of products, waste and residues from biological origin from agriculture and forestry, including plant and animal matters and related industries including fisheries with aquaculture, as well as biodegradable fraction of industrial and municipal waste. Biomass can be easily converted into usable energy sources such as methane or transport fuels (ethanol and biodiesel). There are various technologies of exploitation of energy from biomass: direct heating, converting heat into electrical energy by converting it to another form of fuel such as liquid biofuels or combustible biogas. It should be borne in mind that the use of biomass for heat energy does not increase the CO_2 content in the atmosphere, does not create the greenhouse effect and does not affect the global climate change.

Most of the energy in the world is produced from fossil fuels, which are enormously consumed and the world is in danger of disappearance due to the depletion of their limited

reserves. Therefore, the sources of renewable energy are becoming more and more important. The European Union member states have committed themselves to ensure 20% of share of renewable energy in total energy consumption in the EU (2009/28/EC) by the year of 2020. Biomass will have a significant contribution to achieving this goal. Since the "green" energy produced from renewable energy sources is more expensive than the energy from fossil fuels, special measures are necessary to encourage its production, which is usually done through incentives for collection price or tax incentives for investing in renewable energy sources (Tica et al., 2012).

Biomass can be divided into four groups: wood biomass (sawdust and scrap wood processing), residues from agriculture (straw, corn stalks, husks, pits, etc.), animal wastes and residues and biomass from waste (garbage). The focus of further research is the biomass of plant residues from agriculture or energy production from this biomass. The assessment of the energy potential of biomass was researched by numerous authors, analyzing the situation in different countries, for example Scarlat et al. (2007) for the new EU member states and candidate countries, Liu and Farmer (2010) and Gallagher et al. (2011) in the USA, Stiehler et al. (2011) in Germany, and Jasiulewicz Janiszevwska (2012) in Poland, Jing et al. (2012) in China and Glithero et al. (2013) in England. The selection of equipment for biomass power plant is based on the proper selection of the cogeneration facility. To make a decision on construction of such a facility, all relevant facts should be considered and market, economic, environmental and social justification according to the laws should be checked, as well as the conditions in the energy market.

Materials and methods

The subject of the research presented in this paper is the possibility of building a biomass power plant that would use crop residues from agriculture as fuel.

The objective of the research is to test and prove the economic justification of investment of 967,504 KM (remark: 1 KM = 0.51129 EUR) in the construction and use of biomass power plant of 250 kW. The dynamic methods of evaluation of the investment efficiency are used: payback period, net present value and internal rate of return (Vaško, 2001; Orsag and Dedi, 2011).

(Discounted) Payback period method (p) – time period when investment cost (I) and cumulative discounted net cash flows (Vt) equals:

$$I = \sum_{t=1}^{p} Vt$$

Net present value method (NSV) – the difference in net cash flow (Vt) discounted by discount rate of 8% and subsequent investment (I):

$$NSV = \sum_{t=1}^{20} \frac{Vt}{(1+d)^t} - I$$

Internal rate of return method (r):

$$\sum_{t=1}^{20} \frac{Vt}{(1+r)^t} = I$$

The case of establishment of a biomass power plant in the municipality Pelagicevo has been analysed. Municipality of Pelagicevo is located in Posavina, in the north of Bosnia and

Herzegovina, it covers the area of 178 km^2 and is mostly agricultural. Technical and economic parameters of analyzed investments are shown in table 1.

Table 1: Main technical and financial parameters of the project

Project title	Solid biomass power plant in Pelagicevo	
Project location	Pelagicevo	
The power of plant	kW	250
The annual output	kWh	1,500,000
The value of investments in KM (inclu VAT)		967,504

Results and discussion

Energy sector in BaH has enormous development potential. For a number of years, BaH is the only in the region with a positive balance of imports and exports of electricity, and it is the eighth country in Europe in terms of hydropower potential, which currently uses only just a little bit over one-third (38%). Moreover, it is estimated that BaH has significant potential to produce energy from renewable sources (wind, solar, biomass and geothermal energy), according to some indicators, these potentials are 30% higher than the EU average (Energetski institut Hrvoje Požar, 2008).

The prerequisite for the implementation of the investment project is to determine the incentives of electricity generation from renewable energy sources in terms of the right to a guaranteed purchase price ("feed-in" tariffs) in terms of article 6 of the Rulebook on promotion of electricity generation from renewable sources and efficient cogeneration (Offical Gazzete of the Republic of Srpska, 2013), or guaranteed purchase/collection price of 0.2413 KM/kWh.

Procurement Market

Given the projected capacity of biomass power plants, the procurement market of raw materials (biomass) is limited only to the municipality of Pelagicevo. In the municipality, there is about 9,600 ha of arable land, of which (only) 3,600 ha is cultivated. About 3,000 ha of arable land is under grains and corn, and after the harvest there remains about 12 thousand tons of biomass, of which up to 30% can be used to produce electricity and heat. Huge potential for biomass production is in uncultivated areas, due to the fact that with the clearing of undergrowth or mowing of wild plants, significant additional amounts of biomass can be provided, at low cost. In addition, 1,515 hectares are under forest, and after the whole planning and regular cutting, there are remains of wood residues (small branches), which are generally not used because they are unsuitable for heating. All this confirms that for the biomass power plant in Pelagicevo there are sufficient raw materials in the area, and the municipality (about 2 thousand tons of biomass are annual needs of power plant), and in case of the lack of it, the additional quantities could be purchased from the territory of neighbouring municipalities; Donji Žabar, Šamac, Gradačac and the Brčko District; and without additional transport costs.

Sales Market

It is estimated that the demand for energy in the world by 2040 will be doubled (US Energy Information Administration, 2013), so the sales market of products, whose production is subject of investing, is characterized by growing demand. The increase of world electricity production, in addition to hydro potential, a significant share of other renewable sources, including both biogas and biomass is anticipated. According to the Bosnia and Herzegovina Energy Sector Study (Energetski institut Hrvoje Požar, 2008), the use of electricity in BaH by

2020 will increase by 50%, with projected reduction in the share of individual households use in total use to 41%. The price of electricity in BaH (in 2013 it is 0.080 EUR/kWh) is the lowest in Europe (EU-28 average in 2013 was 0.199 EUR/kWh) (EUROSTAT, 2015). Generally, from the standpoint of sales market in the future, we can expect an increase in the use of electricity and an increase in its price, in Bosnia and Herzegovina, and the region as well as in Europe.

Technical and technological analysis

In Bosnia and Herzegovina, especially in Posavina, remains of grains are of a specific importance for the production of energy from renewable sources. Biomass ratio of cereals (maize, wheat, barley) is 1:1, i.e. the weight of biomass is equal to the quantity of grain (Brkic et al., 2007). The plant residues must be primarily returned to the soil and ploughing between 30 and 70% of this mass is recommended, which means that for the energy application, at least 30% is available. In the municipality of Pelagicevo, at the area of about 3,000 ha, 3,543 tons of wheat, 8,383 tons of corn and 98 t of barley are produced (seven-year average, 2007-12) (Statistical Yearbook of the Republic of Srpska, 2011 and 2013). Electricity produced from biomass would be produced by gasification process in the fixed-bed gasifier with a dual flame of installed power of 250 kW. The total estimated electricity production of this power plant annually amounts to 1,500,000 kWh, and based on the installed active power of solid biomass electric power plant of 250 kW, and 6,000 hours a year work of a power plant.

The structure of investment in a biomass power plant

Investments in biomass power plant consist of the investments in the procurement of fixed assets and the amount of required permanent working capital in total amount of 967,504 KM (approximately 495,000 EUR). They consist of the following: investments in the procurement of construction land, construction of the facility, initial investment (projects, approvals and permits), the construction of substations and procurement of equipment for power plant. The own funds of the investor are 392,524 KM (40.5%), loan 500,000 KM (51.7%) and the EBRD loan subsidy for energy efficiency of 75,000 KM (7.75%). The loan is for the period of 5 years with a grace period of 12 months and interest rate of 8%.

SWOT analyses

The main strengths, weaknesses, opportunities and threats of this investment are summarized in the following table.

Table 2: The project SWOT analysis

Strengths	*Weaknesses*
unused biomass around the power plant; available equipment and technology for biomass power plant; contribution to the environment protection,	lack of knowledge and experience in the production of energy from renewable sources; insufficient own capital; dependence of raw materials suppliers.
Opportunities	*Threats*
growing consumption and demand for electricity; expected growth of electricity price; obligation to produce more energy from renewable sources.	weakly accessible and expensive credits; lack of incentives for the production energy from renewable sources; the risk of changes in legislation (guaranteed quantities and prices).

Economic and financial analysis

Economic and financial analysis of the investment in a power plant using biomass in Pelagicevo was conducted for a period of 20 years, although this investment, with proper periodic maintenance, can bring benefits and revenues for significantly longer period. Because of amortization of equipment, in the year 10, it is envisaged to replace the system for biomass gasification and gas generators for biomass. It would include the investment of 547,771 KM, and the same should be done in the year 20, if the production of electricity from this power station is to be continued. Amortization calculation was made according to annual depreciation rates prescribed in the Regulations on the application of the Law on corporate income tax (Offical Gazzete of the Republic of Srpska, 2006). Total investment expenditures are the following: material costs (biomass), the cost of service, salary expenses, depreciation and cost of financing (interest). Annual expenses are highest at the beginning (322,791 KM) and the lowest at the end of the period of investment exploitation (274,969 KM). Revenue is derived from sales of 1,500,000 kWh of electricity at the price of 0.2413 KM/kWh and are the constant during all these years (361,950 KM). The annual gross profit is 39,259 KM at the begining, and 86,981 KM in the end of the period.

Income Statement is modified into net cash flow of the project of solid biomass power plant in Pelagicevo, and it receives (returns) invested money from the sale of electricity. Since the analysis was performed for the first 20 years of the investment, and the equipment (which will be replaced after 10 years) and the facility can be used much longer, in the end of the year 20 the land, buildings and equipment have residual (liquidation) value of 150,947 KM.

Cash outflow of solid biomass power plants in Pelagicevo are the investments into fixed and current assets at the beginning of the project, and subsequent operating costs of the power plant. The expenditures in the cash flow include the repayment of the interest and all the payments to the state.

Project evaluation by dynamic methods

With the discounted cash flows of the project of solid biomass power plant in Pelagicevo by the discount rate of 8%, its net present value at the end of the year 20 is positive and it is 90,213 KM. Disccounted payback period is 18.08 years. Internal rate of return of the project is 9.19%.

The parameters of dynamic evaluation of investments in the biomass power plant indicate that this investment is on the borderline of profitability. Similar results in the case of the production of pellets from straw were made by Zekic et al. (2014), who concluded that the production of pellets from straw is uneconomical. The economics of biofuel production is closely linked to the price of fossil fuels. Since biofuels are more expensive, their production is worth only if the government subsidizes part of the cost.

Analysis of sensitivity

For discounting, discount rate of 8% is selected, which is closest to the price of the external sources of financing (interest rate=7.75%). Additional analysis was done for alternative discount rates, the lower of 6% and 7% and higher of 9% and 10%, which are in the range of most common interest rates on investment loans in BaH. Understandably, internal rate of return remains the same, and only NPV and payback period are changed.

Table 3: Results of analyses of sensitivity to the change of discount rate

Interest rate	*6%*	*7%*	*8%*	*9%*	*10%*
NPV (KM)	278,069	177,863	90,213	13,229	-54,662
Payback period (years)	15.20	16.48	18.08	19.75	23.24

Analysis of sensitivity of the project showed that lower discount rate (e.g. as a result of concessional credit conditions) improves efficiency of the investment. On the other side

discount rate of 10% significantly deteriorates the image of the investment in the solid biomass power plant, because its NPV is negative and the payback period is extended to 23.2 years.

Socio-economic assessment of the project

In addition to individual benefits for investors, the project brings environmental and social benefits in terms of increased production of electricity from renewable sources. Other social benefits from the realization of the investment of the project of solid biomass power plant in Pelagicevo (shown on the twenty-year level) are: employment of 4 workers; higher capacity utilization at the suppliers of equipment and services on the basis of increasing their production and sales; the environmentally sound disposal of plant residues in agricultural production; providing additional income of 2.5 million KM for farmers from sale of straw; taxes on purchased equipment; income tax of about 145 thousand KM. The project has a special contribution to the municipality of Pelagicevo, which belongs to underdeveloped municipalities of the Republic of Srpska. The widest social contribution of this project is to reduce pollution of the atmosphere

Conclusion

Biomass, as a renewable source of biological origin, becomes more interesting and more frequently used, worldwide. One of the forms of biomasses, is biomass from plant residues from agriculture, which, among other things, can be used for production of electricity.

Analysis of cost effectiveness of investing 967,504 KM in construction solid biomass power plant in Pelagicevo confirms that such investment is profitable, but in the long terms, because of the following:
Twenty-year net present value with a discount rate of 8% is positive (NPV=+90,213 KM);
Invested money is returned to the investor after 18 years (p=18.08 years), i.e. before the expiration of the technological life of the plant;
The rate of return is higher than the cost of capital which financed the project (r=9.19%).

The sensitivity analysis shows that the project could not endure the discount rate (and thus the price of capital) of more than 10%, because the NPV becomes negative, and the payback period exceeding 23 years. Compared to other investment alternatives, the potential investor will certainly think of projects that may bring a faster return on invested funds and higher net present value than the analyzed investment in electricity generation from biomass. It would therefore be neccessary to provide greater incentives for this type of investments, in order to have greater use of biomass for generating electricity, with simultaneous promotion of the benefits of using this type of energy and increase its use.

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A STUDY OF BULGARIAN DAIRY FARMS PROFITABILITY BEFORE AND AFTER ABOLITION OF MILK QUOTAS

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Abstract

The milk quotas were first introduced in 1984 under the Dairy Produce Quota Regulations which reflects the Common Agricultural Policy CAP of European Community (EC). Originally, they were run until 1989 but have been extended several times. The quota system was implemented in Bulgaria in 2007 according to accession treaty with the EU.

The system provided quota rent for all farmers in the dairy sector, however the most advantaged were less productive and efficient holdings. The average productivity per dairy cow in Bulgaria is less than 60% of EU average, which poses Bulgarian dairy farmers in more vulnerable position when policy is changed. A tentative view indicates that dairy production after the quota removal is likely to contract in several member states including Bulgaria which makes the topic actual.

This paper studies profitability of Bulgarian dairy farms after abolishment of milk quotas. Based on developed econometric model authors make an assumption about decrease in farm profitability in 2015 and 2016.

Keywords: *abolition of milk quotas, dairy, farm profitability, Bulgaria*

Introduction

The milk quotas were first introduced in 1984 under the Dairy Produce Quota Regulations which reflects the change in CAP and Common Market Organization of EC. This new policy for milk replaced price support system. In the years before quota system price stimulus caused increase in dairy milk production leading to milk surplus and inability of intervention system to handle the produced volumes (produced volume was with 20 % more than quantity common market can absorb and expenditure on dairy support become the single biggest item in the CAP budget). The aim of the quotas was to contain the production as each member state and each dairy farm has reference quantity, based on production in 1981 plus 2 % of volume. But that rule was flexible for some countries taking other years as base (for Ireland it was 1983). There was also correction in the quota if milk fat level is more than reference level. If quantity exceeds set quota a super-levy payment was due according to Donnellian (et al.), 2014. Originally milk quota system was intended to run until 1989 but have been extended several times until 31 March 2015.

The system of milk quotas has been implemented in Bulgaria since 2007 after accession to the EU according to Regulation (EC) 1234/2007. Since then Bulgarian dairy farms are under pressure. They have to comply with new, higher hygiene standards imposed by the EU and also with increasing competition from imported raw and processed milk (SMP, WMP, condensed milk). The average productivity per dairy cow in Bulgaria is less than 60% of the EU average, which poses Bulgarian dairy farmers in more vulnerable position. The quota was not a binding factor for specialized holdings because between 2007 and 2013 it had never been reached. The highest achievement was in 2008/2009, for both produced milk 88.5% and direct deliveries 71.2%. In the following years quotas had been increased, but quantity of purchased milk had decreased. (MAF, Agricultural Report) Milk quotas were freely tradable

but prices did not change during the examined period. The presented study examines the profitability of specialized Bulgarian dairy farms based on Farm Accountancy Data Network (FADN) data and makes an extrapolation for years after quota (included 2016) based on econometric model.

A tentative view indicates that dairy production after the quotas is likely to contract in several member states including Bulgaria (A detailed study can be found in report "Smooth Phasing-out of the Milk Quotas", 2014) Because of its binding characteristic after removing quotas there is an incentive to increase production for countries where production was limited. According to Donnellian (et al.,) 2014, expansion of production should take place in lower cost regions (with mild climate) not constrained from competition of other agricultural sectors for environmental factors. Countries where production is likely to expand are Ireland, Austria, Belgium, Denmark, Germany, France, the Netherlands and Poland. According to an external report for EU commission (Ernst &Young, 2013) price volatility constitutes one of the greater challenges that dairy producers will have to face in the near future.

Materials and methods

The difference between farm gate milk price under quota and marginal cost for production is described as quota rent. The quota rent cannot be evaluated directly, because there is no economic indicator to measure it. But when quota is tradable its opportunity cost is available as price farmers are willing to pay for additional quota. (For a more detailed theoretical explanation and modeling please read "Economic analysis of the effects of the expiry of EU milk quota system" by Bouamra-Mechemache et al., 2008). Reduction in price volatility and inability for EU producers to avail from international marketing opportunities due to rising global demand for milk and milk products are other effects of the quotas (Donnellian et al., 2014)

Our work hypothesis claim: quota removal will decrease profitability of specialized milk holdings due to increase of the volume of production and competitiveness in the EU milk market, which is likely to lead to a price decline.

Development of profitability of Bulgarian dairy farms is explored on farm level for the period from 2007 to 2016. The approach we use is similar to that used by Van Berkum (2009) exploring the gross margins of dairy farms in new EU member states and candidate countries (revenues minus the cost of variable inputs). We add subsidies to revenues.

An econometric model was built in order to evaluate the profitability after quota abolition due to continuance of time series (until 2012). It is a non-spatial, dynamic, system of equations, where the solution estimates the average profitability per farm. Model has two types of specifications:

Mathematical, based on models developed for Forecast Support Group of Economic Research Service (ERS) of US in the 1970s from Ezekiel, Foote, Fox, Nerlove, Waugh, (Judge, 1977) and a consortium of econometric modeling scholars: Johnson and Rausser (1982). Variables used into the model are divided into exogenous and endogenous (calculated in the model), such as elasticity, regression coefficients and intercepts. Values of elasticity are positive.

Logical, it constructs model based on logical and regression relationships between variables, thus constituting the system of model equations. GDP Deflator[17] was used to reflect the growth of GDP. Government policies on national and EU level are shown by subsidies. An estimation has been made for subsidies based on expected average number of dairy cows per holding and area applicable for support[18]. We expect process of concentration of milk production will continue therefore average farm herd size will grow. Influence of external to the milk market factors are represented through price of raw milk.

Limitations: The length of time series that we receive from FADN database is too short. Some costs as Other livestock specific costs and some specific types of support as LFA subsidies are not included.

Dairy farm income (DFI) is calculated in equation (1) using: Cows‘ milk and milk products (CMMP), Total subsidies (TS), Feed for grazing livestock (FGL) and Total external factors (TEF). TEF is compound variable from FADN database which includes external costs for the farm - wages paid, rents paid and interests paid.

(1) DFI = CMMP + TS - FGL - TEF

We can make suggestion for TS and in order to solve (1) we have to construct modeling equations for CMMP, FGL, TEF. We are applying regression to find significant relations between variables.

Table 1. Regression analysis

Independent variable	CMMP	TEF	FGL
TS	0.61	0.85	0.60
GDP Deflator		0.74	

Significance F is below 0.07. We do not apply regression to all the variables because some are interdependent like average number of Dairy cows (DC) and FGL. Based on regression coefficients in table 1 model equations can be constructed, as follows:

(2) $CMMP = f(x_1, x_2) = \alpha_1 + \varepsilon_1 \beta_1 x_1 + \varepsilon_2 \beta_2 x_2 + \xi_1$

(3) $FGL = f(x_1, x_2, x_3) = \alpha_2 + \varepsilon_3 \beta_3 x_1 + \varepsilon_4 \beta_4 x_2 + \varepsilon_5 \beta_5 x_3 + \xi_2$

(4) $TEF = f(x_1, x_4, x_5) = \alpha_3 + \varepsilon_6 \beta_6 x_1 + . \varepsilon_7 \beta_7 . x_4 + . \varepsilon_8 \beta_8 . x_5 + \xi_3$

where:

α- intercept
β - regression coefficient
ε - elasticity, $0 < \varepsilon < 1$
ξ - factor error

Variable x_1 represent total subsidies (TS). It is compound variable and consists of sum of Subsidies Dairying and Single Area Payment from FADN database. Variable x_2 represent

[17] GDP Deflator is a ratio of nominal GDP to real GDP in the country. Deflator includes only domestic goods and not anything that is imported and it is a measure of the prices of all goods and services.

[18] Subsidies are fixed as amount and are dependent from the number of applicants in any specific year.

Raw milk price (RMP). Variable x_3 represent number of DC. Variable x_4 represent CMMP. Variable x_5 represent GDP Deflator.

Data used: FADN Public Database, Dynamic Reporting Tool (typology TF8, group 5 - specialist milk producers); reports of Ministry of Agriculture and Food (MAF) of Bulgaria; reports of Bulgarian National Statistical Institute (NSI). Values for GDP Deflator and milk price projections for 2015 and 2016 are taken from Milk model of Bulgarian dairy sector developed under project "Creation of Center for Agri-Policy Analysis (CAPA)“ in the Institute of Agricultural Economics (IAE) in cooperation with Food and Agricultural Policy Research Institute (FAPRI), University of Missouri-Columbia. Model of farm profitability was developed using MS Excel 2007 software.

Results and discussion

Modeling results in table 2 shows that the incomes of dairy holding increased after accession of the country to the EU. An exception is identified for 2010, when there is a significant decrease, which can be explained by world financial crisis complemented by a decrease in milk price. The profitability trend during the quota period (from 2007 to 2014) slightly increase attributed as to enhancement of the payments and aids received by farmers and the higher purchase price of milk. It should be mentioned that the purchase price of milk in Bulgaria lags compared to average EU milk price. In 2007, the gap between Bulgarian and EU average milk price is about 24%, which through next year gradually declines, as in 2014, the EU average annual milk price is 16% higher than price obtained by Bulgarian farmers.

The reasons for price divergence are different, as the main one is prescribed to differences in the quality of milk and milk characteristics. The standard employed by dairies in Bulgaria is to buy milk with fat content at 3.6%, which is less than common milk delivery practice found in other member states. Another issue is the quality of milk delivered by dairy farm in Bulgaria, as nowadays also and more severe in previous years a significant part of milk does not meet the EU standards. Nowadays, about 50% of cow milk is provided by farms incompatible with the EU standards, as up to 20-25% of that milk is delivered to dairy establishments.

Results illustrated in Table 2 indicate dairy holdings profitability after quota abolishment will fall probably by 13% on average for 2015 and 2016 compared with the period 2007-2014. It happens despite a rise in subsidies, which farmers will eventually get after the implementation of new CAP 2020. In new CAP 2020, the coupled support designated to dairy production will be heightened by almost 80% in comparison with the average support in the period 2007-2013. The estimated aggregated aids per head in 2015 and 2016 will be very similar to the payments received by farmers in 2014. The income decrease assessed after quota removal is primarily due to the fall in prices of raw milk. The raw milk in 2015 and 2016 is expected to decline by 11% and 6% in 2016 respectively. Those calculations are taken as exogenous from CAPA dairy model, representing the partial equilibrium model of Bulgarian dairy sector. In this relation it is interesting to investigate the role of quota removal in the assumed price fall, which is also prompted by the ban placed by Russia for import of dairy products from EU countries. Of course, the slowed and unstable demand of dairy products in 2015 also contributes to this decline of milk price and reveals the huge uncertainty on the dairy global market. Regarding the influence of Russian ban, Bulgaria is indirectly affected, as the direct export to Russia of dairy goods is very minor but Bulgarian farmers and dairy companies are impacted by the austerity pressure of cheaper dairy products coming from other EU countries re-allocated to other markets after the closure of Russian market.

It is not possible however to make precise evaluation of other factors leading to price decline currently because it is hard to isolate their influence on the milk price. The reduction of milk price inevitably will eventuate to a drop in the farmers' incomes and profitability. Farmers will face profitability cut and it will exacerbate in case they do not boost up their productivity and costs efficiency. The good news for farmers is that there is a lot of room to hoist up the productivity and milk yield, which may safeguard farmers' incomes during next years, when the market pressure from quota removal and market uncertainty will dominate and unleash. But we have to take into account that we study specialized dairy holdings on average, there will be more profitable and less profitable farms. These conclusions confirm our hypothesis.

Table 2 Modeling results

Year	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
CMMP	7771	9146	11325	10297	12528	12048	12306.9	12841.3	12183.1	12270
TS	406	1062	1204	1056	1200	2002	2505	2486.2	2428.5	2583.8
FGL	4590	5568	7107	7357	8401	8265	8368.0	8621.0	8358.5	8912.8
TEF	817	1682	2562	2237	2331	3059	3092.8	3176	3104.6	3244
DFI	**2770**	**2958**	**2860**	**1759**	**2996**	**2726**	**3351.2**	**3530.3**	**3048.4**	**2697**

Conclusions

The study shows that profitability of average farm after abolition of the quotas will probably diminish to some extent, which is mainly attributed to a lower worldwide prices expected and projected. The increase in subsidizing related to coupled aid and area based payments is very likely not to offset the outrunning growth of operational and nonspecific costs in the future, because we assume that holdings will specialize in milk production and biggest part of feeding will be procured externally. The price of grain feeding which is the major part of the cow's diet is assumed to grow slightly in 2015 and 2016 compared to 2014. Despite the price of coarse feeding in the projected years (2015, 2016) is expected to decline slimly the shift to most purchased feeding input will push the operational costs up.

We can expect that some of the farmers in the lower part of the profitability bias will leave the sector. Therefore, the issue with efficiency becomes very relevant and vital, as improvement in the productivity and lessening the fixed and specific costs per cow are envisaged as ultimately important. That is possible if farms invest in pure-breed cattle[19] with higher milk yield and new equipment.

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YOUNG FARMER'S ATTITUDES TOWARDS THE BUSINESS COOPERATION

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Abstract

Business cooperation and networking is one of the basic preconditions for increase economic and social competitiveness of Croatian family farms, and for reviving other economic activities in rural areas. The paper describes some fragmented results from a study conducted among the young farmers in the Croatian continental region. We focused on their attitudes and intention regarding business cooperation in agriculture. The aim of the paper was to describe socio-demographic and professional characteristics of young farmers and to examine whether they perceive the co-operative model as a possible solution to strengthen their business competitiveness. The analysis is based on a survey conducted in December 2014, on a sample of 170 respondents from 18 to 40 years of age. The data were processed by standard statistical techniques for analysis of frequency distributions, percentages and average values, and some pairs of variables have been valued by usual tests (chi-square test and C coefficient). The results indicate that only 49.4% of surveyed young farmers are involved in some form of business co-operation (associations, cooperatives and producer organizations) in agriculture. The majority of respondents (63.9%) consider that the 'cooperative model' is essential for development of agriculture and socio-economic revitalization of Croatian rural areas. Among the group of farmers who operate independently, 45.3% have expressed willingness to be actively involved in establishment of cooperative.

Keywords: *young farmers, attitudes, business cooperation, cooperative*

Introduction

On a global level, agriculture and food sector are facing with numerous challenges such is ensuring food safety (quality and cheap food) in the condition of intensive world's growing population, climate changes and limited natural resources, increasing demands for raw material (biofuels) and sustainable ecosystem management. To overcome these challenges essential forms of collaboration/cooperation in agriculture and in the food value chain are necessary (KPMG International, 2014). Advantages of business and networking in agriculture are in: overcoming individual business constraints and resources, increasing innovation of companies/farms and their products, integrating business operations, reducing/sharing business risk, facilitating access and distribution of business information, promotion of knowledge and innovation, increasing bargaining power, economies of scale and in strengthening position on existing and new markets (Juračak et al., 2000).

According to the social and economic characteristics, Croatian agriculture is mainly positioned on small scale businesses and non-competitive part of agriculture (Nedanov and Žutinić, 2015).[20] These limiting the possibilities of efficient use of agricultural resources for production of healthy local food products and slowdown demographic and economic

[20] In domestic agriculture dominating family farms (96% - 2014) with limited production capacity and inadequate production structure (small holdings, prevailing of low profit activities, small and fragmented production, etc.).

revitalization of the Croatian rural area. Business cooperation and networking are one of a basic precondition for increasing economic and social competitiveness of family farms and for reviving other economic activities in rural areas.
The paper describes partial results from the research study about business cooperation among young farmers in the Croatian continental region. The purpose of the project was to estimate the current situation of business cooperation in agriculture and to gain insight into the perception and preferences of young farmers regarding entrepreneurship and business synergies.
The systematic research on the farmer's business collaboration has been conducted in relatively few. Relevant literature is mainly focused on formal cooperation within cooperatives, producer organizations, business networks and others (Wilson et al., 2014). Gramzow and Petrick (2007) on the examples of post socialist states emphasize the lack of experience and management skills as a common reason for unsustainable joint entrepreneurial farmer initiatives in the agricultural sector. De Regt and Vuylsteke (2011) argued that insufficient aligned of economic policy and institutional framework often resulting with long and complex process of obtaining state aid support which in practice slow down potential of business cooperation among agro-holders (de Regt and Vuylsteke, 2011: 14).
Due the role in the revitalization of EU agricultural sector, issue of engaging the youth in/and entrepreneurship is frequently topic of experts in political discussion, analysis and formal document. However, empirical evidence of entrepreneurial characteristics and intention among young farmers regarding business cooperation are poor.
The aim of paper is to describe socio-demographic and professional characteristics of young farmers and to examine whether they perceive the co-operative model as a possible solution for strengthening their business competitiveness.

Materials and methods

In compliance with EU support within *Young Farmers schemes* (2015-2020), the criterion variable to determine research sample is respondent aged (no more than 40 years old). The sample was composed on collected addresses from the database of the *Croatian Association of Young Farmers* and *Extension Service*. The survey was conducted in December 2014, through the face to face and online questionnaire surveys in continental Croatian regions. The sample included 170 young farmers which are equally distributed through the 14 counties.[21]
For the purpose of paper, the socio-economic characteristic and set of statements about business cooperation in agriculture are included in our analysis. A Likert five-level scale (5= strongly agree to 1 strongly disagree) is used to measure degree of agreement with relevant statements. The data were processed by univariate analysis to measure frequency distributions, percentages and average values, and for some pairs of variables have been valued by usual tests (chi-square test and C coefficient) at level of significance 5% ($p<0.05$). Data were analyzed with the SPSS software package.

Results and discussion

Basic socio-demographic and economic indicators

A summary description of socio-economic characteristics of young farmers is given in Table 1. Majority of the respondents is male (81.9%) who traditionally appears in Croatian agriculture as a sole legal land owner and other productive resources. This is also confirmed by this research where just 4 respondents are young female single holder (total of 44 respondents).

[21] According to Farm Register (2014) Croatian had recorded total of 132.88 family farms, while only 14,078 (10.6%) of holders have less than 40 years. This study included 1.2% of these farms.

Table 1. Basic socio-demographic indicators of respondents (N = 170)

Sample characteristics		N	%
Individual characteristics			
Sex	Male	139	81.8
	Female	31	18.2
Age	18-30 years	89	52.4
	31-40 years	81	47.6
Education level	Basic school	7	4.1
	Secondary school	99	58.2
	High school or university	64	37.7
Marital status	Single	78	45.9
	Married	92	54.1
Solo holder	Man	40	23.5
	Female	4	2.3
Farm characteristics			
Holdings with utilised agricultural land (in ha)*	No land use i < 1 ha	54	31.8
	1 - 10	54	31.8
	10.1 - 50	37	21.8
	≥ 50.1	16	9.4
Main activity on the farm	Crop production	112	65.9
	Animal production	36	21.2
	Mixed crop and animal production	22	12.9

** includes owned and leased farm land; difference to 100% include respondents who didn't answer on this question.*
Source: Questionnaire Survey – own research (2014)

The low farmer level of (formal) education and insufficient of agricultural skills is one of the major development obstacles of family farming sector in Croatia (Žutinić and Markovina, 2009). Data of formal education of respondents indicated that they are significantly better educated than the averaged Croatian farmer. The majority of them (95.9%) have completed secondary school, college or university, while 73.5% of them have some kind of professional education in the field of agriculture (e.g. courses, agricultural school or faculty). Furthermore, 95.9% of respondents are computer literacy and using e-mail and internet for business purposes.

According to the research results, the average farm of young farmers used 17.2 hectares of agricultural land which is significantly above national average (2012 – 5.6 hectares), but at the range between 0.3 and 150 hectares. Two-thirds of surveyed farmers have crop production (arable farming, vegetables in greenhouses, horticulture, viticulture, olive growing, and production of flowers) as a predominant production activity. In livestock production, most of them engaged in the milk production and dealing with cattle fattening. A relatively small number of farms are processing its own products (22.9%). In addition, 29.4% of respondents are providing agricultural machinery services, agritourism (6.3%) and storage services for agricultural products (3.5%). Agriculture is the main source of income for about two-thirds of households (63.5%), 11.2% have the equally income from agriculture and non-agriculture, while 25.3% of them achieves most of income outside the agriculture.

Estimation and attitudes toward business cooperation

The development of business cooperation depends on whether farmers estimate the cooperation beneficial to their business (Kovačić et al., 2001). The survey results show that less than half of respondents (49.4%) are involved in some formal business cooperation in agriculture. In this subgroup (N=84) most of them are members of agricultural associations

(60.7%)[22], cooperatives (11.9%), producer organizations (4.7%) and 22.6% are contractually cooperate with the processing companies. Respondents are estimated the current situation of business cooperation in domestic agriculture as poor and very poor (75.7%, mean=2.12). The main reasons for this situation are „*insufficient knowledge of farmer*“ (mean=4.07), afterward „*low level of awareness*“ (mean=4.03), „*farmer individualism and the importance of personal interests*“ and „*lack of institutional and financial support*“ (mean=3.98).
Almost all respondents (91%) estimating ‘encouraging business corporation’ as a very important fact in national agriculture, especially when we are talking about joint appearance on the market, joint sales promotion and joint processing of their products (Table 2).

Table 2. What aspect of business cooperation you consider as most important?

Aspect of cooperation	Degree of agreement (%)*			Mean	Rang
	1 + 2	3	4+5		
Joining for common land use	51.2	23.5	25.3	2.57	9
Joint production	30.6	32.9	36.5	3.07	8
Shared storage and processing	13.6	18.8	67.6	3.83	4
Joint processing of agricultural products	10,6	20.6	68.8	3.85	3
Joint appearance on the market	3.6	13.5	82.9	4.63	1
Mutual lending	27.7	24.1	48.2	3.24	7
Joint use of agricultural machinery and equipment	15.9	21.2	62.9	3.74	5
Joint procurement of raw materials	22.4	10.0	67.6	3.71	6
Joint sales promotion	5.5	14.7	80.0	4.27	2

* 1=completely not important; 2= not important; 3= neither important, neither not important; 4= important; 5= very important
Source: Questionnaire Survey – own research (2014)

On the question “What form of cooperation is the most acceptable for young farmers?” - the largest number of respondents are emphasize ‘production organizations’ (27.1%), ‘cooperatives’ (25.9%), ‘agricultural associations’ (19.4%), ‘contractual cooperation with processing company’ (14.1%) and ‘clusters of complementary activities’ (5.3%).[23]

Perception of the cooperatives
The recent researches shows that the involvement of the Croatian population in cooperative entrepreneurship is an insufficient and wherefore they have marginal role in the development of agriculture and rural areas (Babić and Račić, 2012; Nedanov and Žutinić, 2015). Therefore one of the research aims was to determine how young farmers perceive cooperatives and are they seen cooperative organizing as a possible solution to strengthen their business competitiveness?
For measuring perception of respondents about the cooperatives we have used 11 statements which are shown in Table 3. Table data illustrates that majority of respondents (78.0%) perceive cooperatives exclusively as economic organization whose business depends on mutual cooperation and the quality of interpersonal relationship. Also, two-third of respondents agreed that cooperatives contributing to the sustainable development of agriculture and rural areas. At the same time, less than half of respondents expressed disagreement with statements that describes the cooperative in a negative context (v5; v8; v9; v2; v7), which can be explained by their inexperience and insufficient level of knowledge about cooperative enterprise model. However, the majority of respondents (63.9%) consider

[22] It is non-profit association of farmers which purpose are to promote agricultural activities and to foster communication and dialogue with administrative departments, experts and scholars in the field of agriculture.

[23] The difference to 100% include respondents (8.2%) whose gives answers: “*it’s better to work alone*”, “*I have a contract with intermediary agent”*, “*I’m not sure, because I don’t have experience”* and other.

that ‘cooperative model’ is essential for agriculture development and socio-economic revitalization of Croatian rural areas.

Table 3. The acceptance intensity of attitudes towards cooperative model

Statement	level of agreement * %			Mean
	1 + 2	3	4+5	
v1. – Co-ops. ensuring better business stability and security for their members.	8.2	28.2	63.6	3.72
v2. - Democratic principles slow and cumbersome operating decisions of cooperatives (1 member - 1 vote).	29.5	32.9	37.6	3.08
v3. – Co-ops. are economic organizations whose business results depend on mutual collaboration and the quality of relationship among members.	3.5	17.6	78.9	4.00
v4. – Co-op. are focused on conservation and sustainable use of local inputs.	11.8	38.8	49.4	3.48
v5. – Co-ops. cannot be efficient because they nourish moral principles and values.	48.8	32.9	18.3	2.62
v6. – Co-op. organizing contributes to sustainable rural development and agriculture.	8.2	25.3	66.5	3.76
v7. – Existence of heterogeneous interests among members can prevented achieving business objectives.	30.6	25.3	44.1	3.22
v8. – Co-op. are traditional organizations who are not open for innovative entrepreneurial ideas and solutions.	49.4	29.4	21.2	2.65
v9. - Co-ops. restrict the freedom of the individual business.	38.2	32.4	29.4	2.98
v10. – Co-op. are increasing trust and solidarity on the village (social capital).	13.5	34.7	51.8	3.49
v11. – Co-op. model is essential for agriculture development and socio-economic revitalization of Croatian rural areas.	63.9	20.5	15.9	2.32

*1= strongly disagree; 2= disagree; 3= neither agree, neither disagree/do not know; 4= agree; 5= strongly agree
Source: Questionnaire Survey – own research (2014)

Contingency analysis (chi-test) has shown that are no significant differences between the socio-economic determinants of young farmers and their perception of the cooperative. Statistically significant differences were observed in only two statement, with respect to the dominant production (v2. p=0.04; Cc=0.39 and v11. p=0.1; Cc=0.43).

Numerous experience around world shows that young people can achieved their own entrepreneurial initiatives and employability by organizing a cooperative. In the subgroup of business independently farmers (N=86), 45.3% of respondent have expressed willingness to be actively involved in the foundation of cooperative, 32.6% do not know, while 22.1% are not interested in this type of cooperation. These shows that the relatively big share of young farmers in Croatia still do not see the cooperative model as a possible solution to overcome their business obstacles.

Conclusion

A summary of the basic research findings on attitudes of young farmers regarding business cooperation in family farming and their perception towards cooperative organizing are as follows:

According to socio-economic characteristic the majority of young farmers (95.9%) are well educated (completed secondary school or higher) and they are computer literacy. The average farm of young farmers used 17.2 hectares of agricultural land and the main farming activities is crop production. Agriculture is the main source of income for about two-thirds of respondents (63.5%).

The survey results show that less than half of young farmer (49.4%) are involved in some formal business cooperation in agriculture. They estimated the current situation of business cooperation in domestic agriculture as poor and very poor (75.7%). More than half of respondent farmers emphasize ‘production organizations’ (27.1%), and ‘cooperatives’ (25.9%) as most acceptable business model for young farmers.

Research analysis of young farmer attitudes towards cooperatives illustrates that majority of them (78.0%) perceive cooperatives exclusively as economic organization. Most of young farmers (63.9%) consider ‘cooperative model’ as an essential for Croatian agriculture and rural development. Among group of business independently farmers (N=86) less than half respondents have expressed willingness to be actively involved in the foundation of cooperative (45.3%)

Long-term empirical research in this and similar fields are required to raise general awareness of the Croatian farmers regarding the benefits of business cooperation.

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CONTRIBUTION OF EXTENSION AND ADVISORY SERVICES TO AGRICULTURE DEVELOPMENT IN EGYPT

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Abstract

Agriculture sector employs about 31% of the labour force and generates 14% of GDP in Egypt. Yet many challenges hinder the development of this vital sector including weak role of extension and advisory services (EAS).

The present paper aims at analysing the contribution of public EAS to agriculture development in Egypt. A review of secondary data was used to give a general overview of the current status of EAS, policy framework and governance structures, main cooperation projects dealing with agricultural/rural EAS as well as human resources and gender issues within EAS context.

EAS played a great role in Egypt's agriculture development for many years. Extension system is currently in a transitional period and is moving towards decentralization of programming decisions and operations from one hand, and being involved in community development, not only agriculture focus, from another hand. EAS face great challenges *i.e.* lack of organizational and institutional coordination, decreasing number of extension workers, difficulty in convincing farmers to change their old and traditional agricultural practices.

Agriculture extension has great potential in achieving sustainable rural development by providing the solutions to field-based problems, improving the income of rural families, enhancing agricultural productivity by increasing input use efficiency and, finally, paying special attention to rural women and youth. It is important to realize that farmers, rural families and rural communities are the ultimate target of EAS. Therefore, all the institutional arrangements for improving, activating and enhancing the mutual cooperation and collective work of the Agricultural Knowledge and Innovation System (AKIS) sub-systems must focus and over emphasize on the characteristics, problems and needs of extension sub-system.

Keywords: *Egypt, Extension and Advisory Services, Farmers, Rural Families, Gender*

Introduction

Egypt is an agricultural-based country, where agriculture incubates about 31% of the labor force (MALR, 2009) and its share in GDP is about 14.5% (CAPMAS, 2006). Yet many challenges hinders the progress and development of this vital sector i.e. deterioration of natural resources, youth migration from rural areas, gender inequality, poor rural infrastructure, weak role of extension services (MALR, 2009).

Agriculture in Egypt witnessed significant developments over the last two decades with direct effects on the role of the agricultural sector in national income formation and promoting exports. Such developments have affected farmers' delivery system as related to the cropping structure, applied technology, levels of income, and farmers' response to market changes (MALR, 2009).

Agriculture sector growth rates have widely differed from one period to another due to the effect of general economic conditions, and development and investment efforts. The 1981/1982 – 1986/1987 period witnessed higher growth, estimated at an annual rate of 3%

during this period, while the 1987/1988 – 1991/1992 period was at an average annual rate of 2% (MALR, 2009). However, in the same time population size increased and reached 72,798,031 inhabitants of which about 41,427106 inhabit rural areas representing about 56.90% (CAPMAS, 2006).

In addition to many threats and challenges that face the agricultural sector and rural areas of Egypt (i.e. small and fragmented land holdings, water shortage and droughts, natural resources and environmental problems, poverty, poor health and malnutrition). High pressures and strains on the country's economy have resulted in poor or weak infrastructure and have pushed rural people deeper into poverty. In this situation, the majority of people is forced to live in poor living conditions and is deprived of basic facilities of life (Mohammed, 2005).

Agriculture extension services played a great role in Egypt's agriculture development for many years, depending upon transfer of information to the public sector. Extension system is currently in a transitional period and is moving towards decentralization of programming decisions and operations from one hand, and being involved in community development not only agriculture focus from another hand. The agriculture extension services face great challenges i.e. lack of organizational and institutional coordination which weakened their role, decreasing number of extension workers, difficulty to convince farmers to change their old or traditional agricultural practices (Shalaby et al, 2011). The number of extension workers in Egypt is 6604 of which 5359 male and 1245 female (Central Administration for Agriculture Extension "CAAE", 2012).

The current study's main objective is to explore the contribution of public agricultural extension and advisory services (EAS) to agriculture development in Egypt. Thus it covers three main themes: overview on agriculture sector, current challenges and limitations; history of agriculture extension in Egypt and its evolution; and role of the Central Administration of Agriculture Extension (CAAE) and its main activities, in order to analyse the current extension challenges.

Material and Methods

In order to achieve objectives, the work was based on extended secondary data review to give a general overview of the current status of extension services, policy frameworks and governance structures of (EAS) in Egypt, main cooperation projects dealing with agricultural/rural advisory/extension services, additionally human resources and gender issues within EAS context.

The main sources were some Egyptian ministries and authorities (*e.g.* Ministry of Agriculture and Land Reclamation, CAAE, Central agency for organization and administration) as well as some international organizations (*e.g.* FAO). The approach adopted is at the same time descriptive and explorative. Various analytical tools were used in order to give comprehensive overview about extension in Egypt, including quantitative and qualitative methods. SWOT analysis was used to verify the current condition of agriculture extension.

Results and Discussion

An overview on the agriculture sector and challenges

Agricultural development efforts during the 1980s, the 1990s and the first years of the twenty first century had achieved great successes in plant production with all its components, due to expanding agricultural areas and improving land productivity. Agricultural areas have increased from around 2 465 400 ha in 1980 to around 3 544 800 ha in 2007, an increase of 44% during this period. The cropping area has increased from 4 662 000 ha in 1980 to 6 468 000 ha in 2007. Livestock/crop production is an excellent example of an integrated production system where fodder crops and agricultural residues provide the feed for animals. The majority of small farmers (about 90% of farmers) follow this system.

More than 95% of the landowners hold less than two hectares each. Only less than 5% own 2.1 ha or more. Almost all livestock are raised through livestock/crop production integrated systems. Crop production includes field crops, vegetables, fruit and forest trees, and medicinal, aromatic and ornamental plants. The annual total cropped area is estimated at 6 468 000 ha, giving a cropping intensity of about 183% in 2007.
Limited land, limited water and rapidly growing population require continuing intensification of production on a limited natural resource base. Moreover, changes in the socio-economic environment have been brought about by changes in urbanization and higher incomes and the need for more export earnings or substitution of imports (El-Nahrawy, 2011).
Agriculture and rural extension is one of the means to alleviate poverty and improve food security, as it promotes transfer and exchange of information to enable and ensure small farmers and rural people accessibility but this is related to developments in institutional systems (Rivera et al., 2001).
Hence agriculture extension is no longer tied to agriculture only or even rural people alone, but extension is a multifunctional process that could be related to health, education, industry, tourism. People in rural areas in Egypt are engaged in farm and non-farm activities, for instance the average annual income of rural families from agricultural enterprises amounts to 4648 L.E. (Egyptian pounds) and about L.E. 2676.5 from non-agriculture activities (CAPMAS, 2012).

History of Agriculture Extension in Egypt

Agriculture extension in Egypt is mainly governmental and it evolved through five stages (Central Administration of Agriculture Extension “CAAE”, 2014).
First stage, “scattered” extension services efforts distinguished this stage among unspecialized groups or organizations i.e. agriculture cooperatives and some commercial institutions, in addition to some visits carried out by engineers working in family farms to provide such services to large scale farmers to aware and help them increase their productivity.
Second stage, extension services were to some extent determined and supervised by various authorities, upon issuing the Village reform act no. 30/1944. This act aimed at establishing agricultural groups covering an area of about 15,000 Feddans to promote agriculture reform and also forming an agriculture council for each group responsible for dissemination of Ministry of Agriculture guidelines, beside providing some technical lectures. This stage was marked in general by the absence of an independent agricultural extension service system and was considered as “secondary” job for some agricultural non-specialized organizations and there were no services targeting small-scale farmers.
Third stage, resulted as a response to solve the problems of the previous stage via unification and independency of the extension system to provide outstanding services within an organizational framework in 1953. This stage was affected by two events, first the extension conference in the Near East held in Syria in 1953, which encouraged the establishment of independent extension services. FAO recommended in the conference to establish agriculture extension centres in the region. The second event was the 1952 revolution which paid attention to agriculture extension as one of the important programs for social and economic development.
Forth stage, agriculture extension evolved between 1953 and 1983 (thirteen years). From 1953 to 1958 agriculture extension was recognized as an official organization in Egypt, by establishing an Agriculture extension department under the umbrella of the General administration of agricultural culture in the Ministry of Agriculture and included four branches at the central level: programs, training and technical services, agricultural units, model fields and cooperatives services, and rural organizations. Whereas at the local level agriculture extension was represented only by a supervisor or an assistant supervisor at the

governorate level along with an extension engineer at the district level (markaz) in the agriculture unit. These agricultural units reached at that time about 62 units.
From 1963 to 1964, the Agriculture extension department became the General administration of agriculture extension and training, and for first time research and extension means were included,
Then between 1964 and 1968 training was separated again and the Central administration for agriculture extension (CAAE) became directly under the supervision of Ministry of Agriculture.
In 1968-1976 the High council for agriculture extension was established by the ministerial decree no. 251/1968 and formed by representatives from universities, high agricultural institutions, farmers, cooperatives, research and services departments related to the Ministry of agriculture. This council played a consultative role to address agriculture problems and suggest appropriate solutions, recommendations to direct extension policy. This decree was followed by another one no. 478/1968 that organizes the General administration for agriculture extension. This period was distinguished by paying more attention to agricultural research and its activities were extended from improving agriculture production to rural developmental activities.
In 1976 -1979 there were additional organizational changes, and this period was marked by the establishment of new directorates to expand extension activities. Within the period 1979-1983 the agriculture extension administration was re-organized by the ministerial decree no. 151/1979 to maximize the agriculture production via educational activities to farmers using demonstration fields and modern visual and audio-visual aids.
Fifth stage, from 1983 till now, agriculture extension administration became under the supervision of the Agricultural research centre (ARC) by the ministerial decree no. 744/1982, to integrate agriculture extension with agriculture research. Accordingly, three divisions were established; agricultural researches, production and stations, and agriculture extension.
In spite of all these efforts, agriculture extension faced many problems regarding the continuous change in its title besides spatial and organizational problems since 1983-1990 until the organized framework and structure assigned by ARC and MALR and approved by the Central agency for organization and administration (CAOA). Though CAAE is still a dependent body at the level of administration.

The role of the Central Administration of Agriculture Extension (CAAE)

CAAE is responsible about reducing the productive gab in various fields to increase self-reliance rate of strategic crops, incorporate both research and extension activities, participate in providing and dissemination of technologies in rural areas regarding production and marketing of field and horticulture crops, besides animal production and rural development. Additionally, CAAE develops new extension methodologies i.e. participatory extension via farmers' field schools (FFS), farmer-to-farmer extension (FTF) and incorporation of population and environment culture within agriculture extension (APEX). CAAE encourages the bottom-up approach in extension program planning in various agriculture production fields, supervision and monitoring extension and training programs in the governorates, responds to farmers' new educational needs within globalization context. Moreover, it is concerned with human resources development through internal and external training, producing agriculture programs and using media audio- visual aids besides participating in local and international exhibitions. Finally, CAAE aims at increasing the productive efficiency of rural families by implementing small-scale projects and providing awareness in nutritional extension and rationalization of consumption, population, health and environmental areas.

CAAE organizational structure, as illustrated in figure 1, is composed from 21 departments and covers nine geographical areas in Egypt. There are specialists, i.e. in rural development (RD), sugar crops, new lands, at both governorate and agricultural units levels, besides extensionists at village level. CAAE possesses 4 information support centers in 4 main areas in Ismailia governorate, in Malawy (Menia governorate), Mariout (Alexandria governorate) and Dekernis (Dakhlia governorate) (Central Administration for Agriculture Extension “CAAE”, 2014).

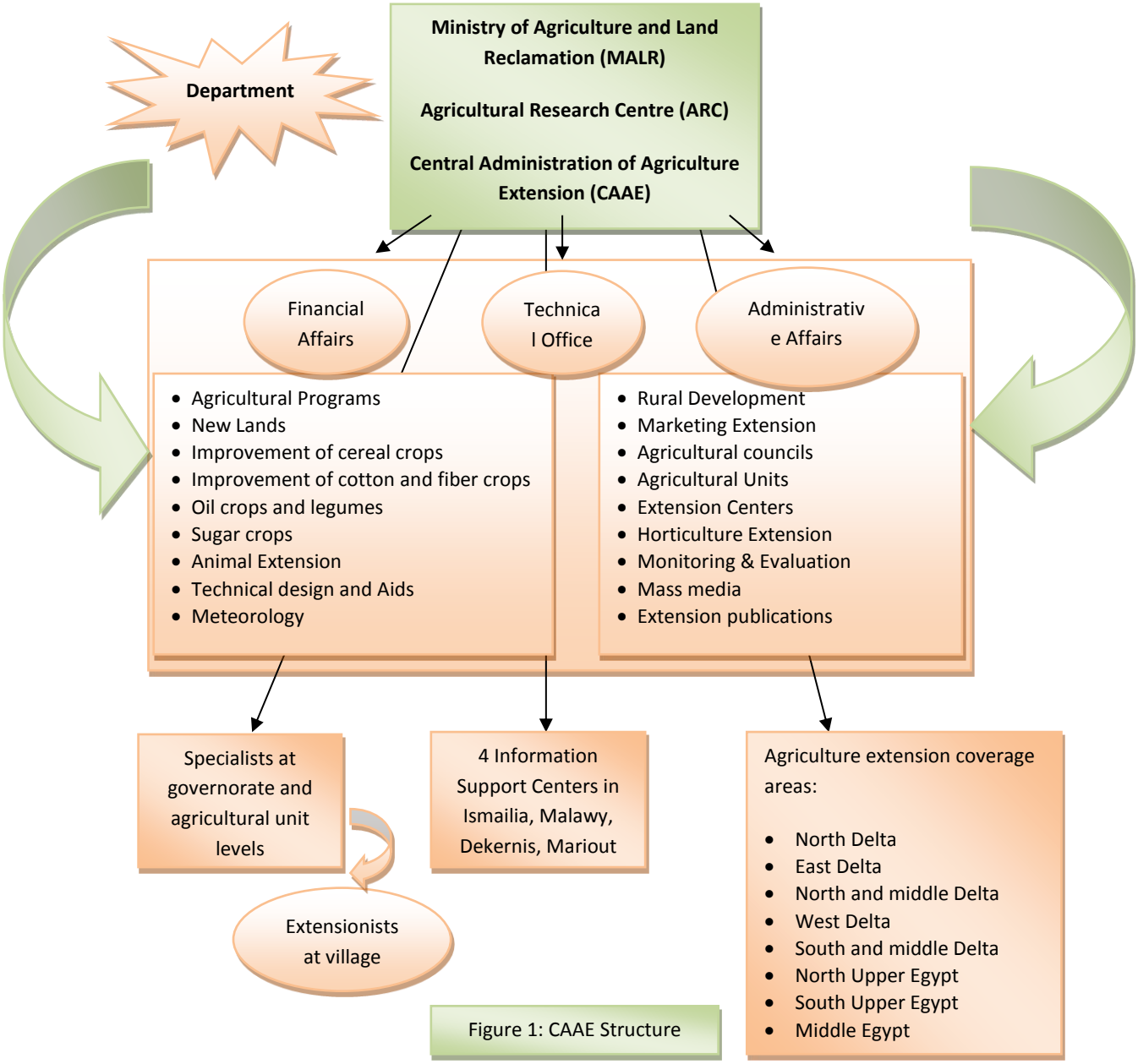

Figure 1: CAAE Structure

Current challenges, expected role and future vision

Egypt’s agricultural extension services had been delivered by vast network of specialized research institutions, central laboratories, regional research stations and extension services. The transfer of knowledge was largely top-down and one-way, from extension workers to farmers.

Extension services faced two main challenges weak links and slow communication between extension and research; and the lack of farmers’ participation in setting priorities.

To address these challenges, the extension units needed to move away from a reactive mode to a more proactive one and to see the beneficiaries of their services not simply as receivers of information, but also as providers of information (FAO, 2010).

There were various and diversified experiences that adopted new approaches in order to face the challenges within the new developments for instance MALR and FAO along with some Egyptian organizations launched two projects tackling ICT in extension and RD were implemented, first the Virtual Extension and Research Communication Network (VERCON) initiated in 2000 and second Rural and Agricultural Development Communication Network (RADCON) initiated in 2004. Both projects aimed at strengthening communication among stakeholders in agricultural and RD, RADCON was built on the experience of VERCON. In addition to farmers field schools (FFS) approach, which is part of long-term strategy of sustainable agriculture development applied by MALR and aims at improving the standard of living of rural inhabitants from gender perspective, by increasing their skills and access to knowledge. FFS was initiated for first time in Fayoum governorate in 1989 to improve tomato crop by establishing 3 female farmers' schools followed by IPM-FFS in 1999.

In the last years agriculture extension faced problems and its role weakened but it still has an important and vital role to play with farmers, rural people and their communities, thus extension services as mentioned no longer depend on agriculture or helping farmers only but to expand and extend their role to cover all people's livelihoods. Therefore, improving crops, animal wealth, poultry, fishery, water resources from one hand and people's life quality and living standards from another hand is what ought to be the coming role of extension in Egypt and in developing countries. In order to evaluate the current challenges facing agriculture extension, SWOT analysis was carried out based on review of literature as illustrated in table 1.

Table 1. SWOT analysis of current agriculture extension condition in Egypt

Strengths	**Weaknesses**
Extension service providers exist either public or private Legislations availability Experienced extension staff Farmers increased demand to improve their conditions via extension services The existence of the Sustainable agricultural development strategy	Low number of extension workers Weak role of agricultural cooperatives No gender balance in extension providers and recipients Budget constraints Extension workers overloaded with reporting for agriculture land violations
Opportunities	**Threats**
Incentives to attract young extension workers Improved coordination and cooperation among relevant stakeholders Training for young extension workers Decentralization with minimum supervision at ministry level	Limited markets access to small-scale farmers Losses in agricultural lands Scarcity and deterioration in natural resources Poverty and food insecurity High marketing competition locally and internationally

Source: Review of literature and CAAE

Conclusions

Agricultural extension and advisory services (AEAS) is one of the tools through which agricultural production and productivity could be promoted. Since improving AEAS in Egypt is essential and urgent, any strategy or policy for enhancing their roles in the promotion of agriculture should be based on achieving the following four main objectives or functions:

1) Achieving food security through technology transfer, to produce more food for satisfying the mounting demands of the ever-increasing population;

2) Human Capital / Resources Development, through improving the behavior (the Knowledge, Attitudes, Aspirations and Practices / Skills - KAAP / KAAS), of each individual producer;
3) Social Capital Development, by getting farmers better organized through two different mechanisms: a) Networking through establishing collaborative relationships in production and marketing, among farmers, and, b) Encouraging producers to form or establish farmers organizations as Civil Society Organizations / NGOs or Community Based Organizations for production and / or marketing of specific commodities or crops;
4) Sustainable Management of the Natural Resource Base, through conserving and sustaining the available natural resources (especially water and land), and avoiding their depletion, pollution or deterioration (El-Shafie, E. M. 2014).
Finally, without a clear strategy and re-structuring CAAE, hiring young extension workers and applying new participatory approaches that assure coordination and integration among research, farmers and extension, then no progress will take place.

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FARMERS' INFORMATION ABOUT THE POTENTIAL IMPACTS OF CLIMATE CHANGE IN BEHEIRA GOVERNORATE IN EGYPT

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Abstract

Climate change is different from most of the other environmental problems. It acquires a universal dimension as it transcends countries national boundaries and constitutes a threat to the entire world. Egypt is one of the vulnerable countries to the potential impacts of Climate Change. Therefore Farmers' information (FI) about the potential impacts of climate change on Egypt (PICCE) is the main question of this study. The objectives of the study were: to measure the FI about PICCE, and to identify the relationship between the "FI about PICCE" and some of respondents' personal and socio-economic characteristics

This study was conducted in Beheira Governorate, which is located northwest of the Nile Delta, near the Mediterranean Sea Coast in Egypt. Field data were collected during January and February 2015 by a pretested form through personal interviews with a sample of 172 farmers. Frequencies, percentages, maximum, minimum, range, and Pearson correlation coefficient were used for data presentation and analysis.

The most important results are as follows: The majority of the responding farmers fall in the medium and high categories of FI about PICCE by 57.6% and 34.3%, respectively. Compared to only 8.1% of them that fall in low category of FI about PICCE. While, the majority of respondents (96.0%) are unaware that these Risks and threats caused by climate change/global warming. Also, statistically significant correlation relationships were found between the respondents' total score of FI about PICCE and household size, size of farm holding, and exposure to information sources.

Keywords: *farmers' information, climate change, Egypt*

Introduction

Climate change is not a far-off problem. It is happening now and is having very real consequences on people's lives. Climate change is disrupting national economies, costing us dearly today and even more tomorrow. But there is a growing recognition that affordable, scalable solutions are available now that will enable us all to leapfrog to cleaner, more resilient economies (UN Climate Summit, 2014). It is different from most of the other environmental problems. It acquires a universal dimension as it transcends countries national boundaries and constitutes a threat to the entire world. Successive increase in surface air temperature has been confirmed worldwide. Over the past 100 years, the universal average of surface air temperature progressively increased by 0.3 - 0.6°C. The Intergovernmental Panel on Climate Change (IPCC) studies concluded that an increase would, if the situation remained unchanged, lead to serious problems, such as sea water level rise bearing the threat of low laying areas getting drowned. It would also affect fresh water resources and crop production it asserts the spread of many diseases (EEAA, 2015).

Africa is the most effected continent (DMCDD, 2011). As well as Egypt is one of the vulnerable countries to the potential impacts of climate change. There has been a general increase in summer temperatures averaged over the country as a result of human influence on climate (Met, 2011). Given that Egypt's population, land-use and agriculture, as well as its economic activity are all constrained along a narrow T-shaped strip of land along the Nile

and the deltaic coast, it is extremely vulnerable to any adverse impacts on its coastal zones and water availability from the Nile. (Agrawala *et al*., 2004). Egypt's unique geography provides a serious challenge for adaptation to the changing climate and makes change in sea level or the flow of the Nile an extreme threat to Egypt's population and economy (Sterman, 2009). So Egypt faces serious risks from climate change. With 88% of its water coming from the Nile River (the Nile) and 97% of its population living along the Nile River Delta (the Nile Delta), a substantial reduction in flow of the Nile would pose a serious risk to Egypt. In addition, sea level rise (SLR) threatens settlements and agriculture in the Nile Delta and also in the Red Sea. Egypt is already hot and dry. Higher temperatures alone threaten to evaporate more water, increase the need for water supplies, create more heat stress, exacerbate already high levels of air pollution, and drive away tourists (Smith *et al*., 2013)

However, the climate change crisis is still a growing reality, which will require continued response not only from all of us. This global climate crisis calls for an urgent and holistic response, which tackles both the causes and the effects of climate change. Systems, policies, business practices, attitudes and lifestyles need to change – primarily in North but also in South – for the sake of future generations. (DMCDD, 2011).Adaptation priorities for coastal zones and the agriculture sector in the adaptation entail: conducting a national program for integrated coastal zones management; improving current crop patterns; developing a calendar to be adapted to the projected climate changes; improving the on-farm irrigation system; and developing a special fund program for adaptation and risk reduction activities for coastal zones and the agriculture sector (EEAA and UNFCC, 2010).

To confront these adverse impacts of climate change on Egypt, the Prime Minister re-established in 2007 a National Climate Change Committee consisting of scientists and experts from different ministries and relevant bodies responsible to manage climate in Egypt and to set a strategy to adapt to potential global changes (Saber, 2009).

Farmers' information (FI) about the potential impact of climate change on Egypt (PICCE) is the main question of this study. Because they are from the most affected groups by these negative impacts, therefore they must modify their behavior to avoid or adapt to these impacts. Therefore the objectives of this study were:

- Measuring the level of FI about PICCE;

- Identifying the relationship between "FI about PICCE" and some of respondents' personal and socio-economic characteristics.

Materials and Methods

This study was conducted in "El-Kanays" village, in "Kafr El-Dawar" District, Beheira Governorate, located northwest of the Nile Delta, near the Mediterranean Sea Coast in Egypt. Field data were collected during January and February 2015 by a pretested form through personal interviews with a sample of 172 farmers, representing more than 10% of the total holders of agricultural land in the village. Frequencies, percentages, maximum, minimum, range, and Pearson correlation coefficient were used for data presentation and analysis.

The dependent variable for this study "FI about PICCE" was measured by a scale consisting of 14 sentences representing the potential impacts of climate change on Egypt in 4 areas: Water Resources, Human health, Agriculture and Food, and Socio-Economic. There were two alternative responses for each sentence; know and don't know; they were assigned with values: 1 and 0, respectively. The total score for each respondent consist of his total responses' degrees about the 14 sentences of the scale. Then the respondents were divided into three categories according to their total scores.

Results and Discussion

The results in table 1 show that: With regard to the farmers' information (FI) about the potential impacts of climate change on Egypt (PICCE) in the area of Water Resources, the majority of responding farmers (63.4%) fall in the Medium category. And regarding the FI about PICCE in the area of Human health, more than a half of the respondents (51.2%) don't know it versus less than a half of them that know it, while the majority of them fall in the medium categories of the FI about PICCE in the areas of Agriculture and Food, and Socio-Economics with percentages 58.7% and 52.3%, respectively.

And regarding the FI about PICCE in all areas, the results in table 1 show also that: The majority of the respondents fall in the middle and high categories by 57.6% and 34.3% respectively. Compared to only 8.1% of them that fall in low category.

Table 1. Distribution of sample by FI about PICCE categories

Information categories	Frequency	%
Water Resources		
High (4)	47	27.3
Medium (2-3)	109	63.4
Low (1)	10	5.8
Don't know (0)	6	3.5
Total	**172**	**100.0**
Human health		
Know (1)	84	48.8
Don't know (0)	88	51.2
Total	**172**	**100.0**
Agriculture and Food		
High (4-5)	36	20.9
Medium (2-3)	101	58.7
Low (1)	16	9.3
Don't know (0)	19	11.0
Total	**172**	**100.0**
Socio-Economics		
High (4)	55	32.0
Medium (2-3)	90	52.3
Low (1)	15	8.7
Don't know (0)	12	7.0
Total	**172**	**100.0**
All Areas		
High (10-13)	59	34.3
Medium (6-9)	99	57.6
Low (2-5)	14	8.1
Total	**172**	**100.0**

Source: Calculated from field data

The results in table 2 indicate: statistically significant and positive correlation relationships were found between the respondents' score of FI about PICCE in the area of Water Resources and formal education years and size of farm holding, while there was a statistically significant and negative correlation relationship with household size. On the other side no statistically significant correlation relationships were found between the respondents' score of FI about PICCE in the area of human health and any of respondents' characteristics. And

regarding agriculture and food a statistically significant and positive correlation relationship was found between the respondents' score of FI about PICCE and size of farm holding. There were statistically significant and positive correlation relationships found between the respondents' score of FI about PICCE in the area of socio-economics and size of farm holding and exposure to information sources, while there was a statistically significant and negative correlation relationship with age.

Regarding FI about PICCE in all areas, the results in table 2 indicate also: statistically significant and positive correlation relationships were found between the respondents' score of FI about PICCE and size of farm holding and exposure to information sources, while there was a statistically significant and negative correlation relationship with household size.

Table 2. Pearson correlation coefficient between the degree of FI-PECC and their personal and socio-economic characteristics.

Respondents' characteristics	Respondents' score of FI about PICCE				
	Water Resources	Human health	Agriculture and Food	Socio-Economics	All Areas
Age	0.032	0.031	0.021	- 0.166*	- 0.050
Formal Education years	0.150*	0.112	0.029	0.006	0.113
Annual income	- 0.009	- 0.019	0.011	0.030	0.013
Father formal Education years	- 0.063	0.047	- 0.073	0.173*	0.029
Mother formal Education years	- 0.073	0.039	- 0.014	0.190*	0.063
Household size	- 0.189*	0.043	- 0.113	- 0.123	- 0.198**
Size of farm holding	0.174*	0.026	0.150*	0.254**	0.226**
Exposure to information sources	0.080	0.113	0.060	0.174*	0.182*
** Correlation is significant at the 0.01 level				* Correlation is significant at the 0.05 level	

Source: Calculated from field data

Conclusion

The findings show generally that the majority of the responding farmers fall in the medium and high categories of FI about PICCE in all areas except the area of human health. Therefore the trend of FI about PICCE level is high. But in the same time 96.0% of respondents are unaware of these negative effects, which are well-known and already happening now in Egypt due to climate change/global warming.

Therefore, there is a significant need to extension development programs and awareness raising campaigns for farmers about these negative effects and their relationship with climate change in Egypt, as well as the importance of adoption the sustainable practices to reduce, face and adapt to these potential risks.

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WOMEN'S EMPOWERMENT AND THE TWINS PRIORITY GOALS: FOOD SECURITY AND HUNGER ERADICATION IN THE MEDITERRANEAN

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Abstract

Today food security and hunger eradication are technically and politically among the two priorities on the international, regional and national agenda due to their impact on agriculture productivity, economic development, food price spikes and climate change. Across developing countries, including those of the Mediterranean, rural women assume critical roles in attaining each of the pillars of food security: availability, access, and utilization. However, their roles are under evaluated and constrained by limitations on their access to resources, services and labor market opportunities. Most of the developing countries of the Mediterranean (lack of data combined with gender - based perceptions) have limited awareness and appreciation of the rural women's productive roles and contributions to food security and fighting the hunger. The inequitable distribution of rights, resources and power as well as repressive cultural rules ad norms, constrain greatly the ability of women to take an effective part in achieving food security and reducing famine expansion. In most developing countries the question which is under continuous bargaining is how to integrate gender across this thematic area? The direct answer which is well recognized should be through the promotion of gender equality and women empowerment in the area of public administration, local governance, human rights and anticorruption. Particular attention should be given to laws concerning property and inheritance. For most developing Countries, it is out of discussion that land ownership is a major determinant of the ability of rural women to improve the productivity of the land they use. However, beside elimination of existing discriminatory legal provision, it is needed to have appropriate strategies at the country level, allocating responsibilities across the appropriate departments - in particular education, health, employment, social affairs, agriculture and rural development. A shift from top down technically driven strategies and programmes to bottom - up participatory ones are urgently needed . Further more social and cultural norms and the gendered division of roles they impose, must be challenged. Empowerment of women is required due to its instrumental value being a condition for society to benefit from the increased contribution of women to food, security, adequate nutrition and poverty eradication.

Key words : *food security, hunger, gender, strategies empowerment, women.*

Introduction

Of the many global challenges we face today, perhaps the most significant and on the longest duration is that of providing food security and eliminating hunger. This challenge is compounded by developments such as the rise and volatility of food prices, the shift from food crops to biofuels in major food exporting countries, the neglect of agriculture in many developing countries, especially in terms of investments in infrastructure, and the looming threat of climate change with its predicted adverse effects on food production.

Persistent hunger and malnutrition is a problem affecting millions of people, the majority of whom are women and girls. Food and nutrition insecurity is a political, economic and environmental issue, but, most importantly, it is a gender justice issue; stark gender inequalities are both a cause and an outcome of unjust food access, consumption and

production. Hunger, malnutrition and poverty are intrinsically human phenomena, yet they are too often discussed in abstract, de-contextualized ways that are far removed from peoples own experiences and fail to adequately understand how they engage in food systems. In particular food security and nutrition programmes often fail to examine the different contributions made by women and men within these systems, and the specific constraints they face: there is also a failure to link experiences of food insecurity and malnutrition on the ground with the gendered inequalities that often lie at the heart of food systems at global, regional, national, local and household levels (FAO, ADB, 2013).

Country examples demonstrate the importance of capacity building, dissemination of information, gender-sensitive statistics, legal assistance, mobilization and advocacy in enhancing women's right, increasing their capability to eradicate poverty, fighting hunger and facing food insecurity. Lessons learned from various contexts that have produced tangible results need to be better shared and good practices scaled up.

Women as food producers

The FAO reports that women comprise around 43 percent of the agricultural labour force in developing countries. The percentage is as high as, or even higher than 80 percent in some countries in sub-Saharan Africa (FAO, 2011): the vast majority of food production that is attributable to women makes them the principle agents of food security and household welfare in rural areas (IFAD, 2009). Gender analysis shows us that women literally feed the world, as producers, processors, cooks and servers of food. However women's vast contribution to food productions, and their key role as consumers and family careers, is still largely misunderstood and underestimated (Sweetman, 2012). Indeed there is little detailed focus on who producing our food, and only a limited amount of gender disaggregated data (UN women, 2013). The available information indicates that women and often girls are heavily involved in all aspects of agricultural productions, processing and distribution. They are, therefore, vital contributors to food security, yet ironically much of their work, both paid and unpaid, is unrecognized. There is also little information on women's specific knowledge and skills in relation to agricultural production, on gender divisions of agricultural labor and on the specific ways in which there is access to productive resource often limited by inherent social and economic norms. Women despite of their vital contribution, they often do not take credit for much of their farming labor and the other household tasks they perform, which directly contribute to food availability and access. Moreover, due to their specific roles in food production, many women are the repositories of knowledge about cultivation, processing and preservation of nutrition and locally adopted crop varieties. Given the right possibilities, such knowledge can allow women to be innovative leaders in sustainable agriculture. Unfortunately, despite their wealth of knowledge and capacity, women farmers are neglected by policy makers, often not being recognized as productive farmers. For sustainability we need long term efforts to increase production, stabilize food availability, and improve distribution. Here the role of women as farmers, as consumers and as family food managers can prove pivotal.

Gendered food production constraints

Women are key players in the farming sector, women's role in food production within agriculture is even greater. In many societies women supply most of the labor needed to produce food crops and often control the use or sale of food produce growth on plots they manage. Improving women's productivity in agriculture not only increases food availability for household but also raises women's incomes and enhances food security due to women spending patterns (FAO, 2011). However, the symmetries in ownership, of access to, and control of livelihood assets such as land, water, energy, credit, knowledge and labor negatively affect women's food production. Women ability for own or inherit land is

restricted in much of in developing countries. Globally women own less than 20% of agricultural land (World conference on Family Farming, 2011).
Insecurity of land tenear for women results in lower investment and potential environmental degradation, it compromises future production potential and increases food insecurity. The lower production reduces not only women's potential income, but, also the availability of food for household consumption. In this connection it is important to focus on gendering land questions instead of treating land issues only in terms of land rights of women (Jakson, 2003). Gendering land questions requires more ethnographic research of social relations around land.
A gender role of women hinders access to technology, agricultural training and rural infrastructure (WB, FAO and IFAD, 2009). Involving young women and men in training opportunities from the start has proved to be a successful strategy in ensuring food security and sustainable livelihoods for households.This is also the case concerning the access of education and new technologies. Women's limited access to technology resulted in failure to address women's time constraints to agricultural activities. However, adoption of new technology depends on many things, including the availability of required assets to implement the technology, how local women and men view the perceived benefits, the way the information is shared, and local gender roles and other socio cultural constraints.
Even when women have access to land for food production and access to improved technology, they face more constraints than men in accessing complementary resources for success. They have less access to credit, and less access to inputs such as fertilizers, and they are less likely to benefit from agricultural extension services and therefore they have less access to improved technology. These constraints are not only costly to food security but also to economic growth (World Bank, 2009). There are numerous studies outlining women's limited access to markets compared with men (Kabeer and Humphrey, 1991; Kabeer, 2012; UNDESA, 2009). Changes in policy and regulatory frameworks are needed to create an equal playing field for women and men in market participation. Other major constraint is that many women in developing countries have less access to resources such as credit and financial capital, that could improve their lives sustainability and facilitate their entry into markets (IAASTD, 2009). Limited access to resources sustainability reduces women's ability to invest in new technology or adopt new agricultural techniques (FAO, 2009).

Gender and food security

Food security, at the individual, household, national, regional and global levels is achieved when all people of all times, have physical, social and economic access to sufficient, safe, and nutrition food to meet dietary needs and food preference for a healthy and active life (FAO, 2003). The "at all times" element of food security definition makes risk and associated vulnerability an important element of the food security concept.
Today the world has enough food to feed everyone, yet an estimated 854 million people worldwide are still undernourished (FAO, 2006 a and b). Poverty not food availability is the major driver of food insecurity, but the two are not always limited. Poorer households headed by women have demonstrated that they often succeeded in providing more nutritional food for their children than those headed by men (Kennedy and Peter, 1992). This demonstrates the importance of greater – based knowledge and roles with regard to food security.
Improvements in agricultural productivity are necessary to increase rural household incomes and access to available food but are not sufficient to ensure food security. Food security not only requires an adequate supply of food but also entails availability, access and utilization by all men and women of all ages. Gender – based inequalities all along the food production chain from farm to plate impend the attainment of food and nutritional security.

Nutritional security requires that household members have access not only to food, but also to health care, a hygienic environment, and knowledge of personal hygiene. Food security is necessary but not sufficient for ensuring nutrition security (IFAD, 2001).
There is differences in food security analyzed on national level with that analyzed at the household one. At the national level, food security implies not only the importance of national production, but, also the country's access to food from the global market, its foreign exchange earnings, and its citizen's consumer choices. Regarding the household food security it is conditioned by household's own food production and household members' ability to purchase food on the right quality and diversity in the market place.

Food security: gender equality and women's empowerment

Provision of equal opportunities for men and women, boys and girls, is necessary for all the full realization of their contribution to sustainable development. Therefore achieving gender equality and women's empowerment is important. In this regard, some areas to be considered include: equal education at all levels and achieving universal access to and quality of education is also important in promoting gender equality and empowerment of women and in shaping values and creating the necessary skilled and productive labor force, further important areas to be carefully considered are: ensuring equal participation of women in decision-making; equal access to assets and resources, ensuring equal employment opportunities for women and equal pay for equal work and promoting gender equality policies supported by gender disaggregated data (WFP, 2009; UN university, 2012).
According to FAO, 2006, the gendered aspects of food security are visible along the four pillars of food security: availability, access, utilization and stability. For FAO, ADB, 2013, gender inequality both leads to and result in food insecurity. According to estimates women and girls make up to 60% of world's chronically hungry and little progress has been made in ensuring the equal right to food for women enshrined in the convention on limitation of all forms of discrimination both in education and employment (Spoeldoch, 2011) and world food programme gender policy report (2009).
Individually as a group, institutions or nation can play an important role towards gender equality by:
Offer education particularly to children;
Increase women employment
Involvement in policies and social activities; and
Arrange social protection programmes.
In addition, there is high potentiality to eradicate poverty and achieve food security through directing further efforts and work towards:
Generate awareness among public and rural society.
Provide possibilities to girls and young women to further their education
In this connection governments can have an important task to challenge the poverty problems and food security by improving policies and adjusting their structures, giving the needed attention to the following five core areas:
Facilitating agricultural markets and trade;
Improving agricultural productivity;
Investing in public infrastructure for agricultural growth;
Reducing rural vulnerability and insecurity and
Improving agricultural policy and institutions.
Looking to rural women's empowerment the future implies that the following issues should be carefully considered:

What are the keys areas where new and interventions are necessary to make measurable and accelerated progress in rural women's empowerment? What gaps need to be addressed? What is the role of different stakeholders?
How can existing policy commitments to rural women's economic, legal, and political empowerment be more effectively translated into concrete actions on the ground? What measures and strategies have been successful and how can those be scaled up?
What current challenges and emerging trends have an adverse impact on the situation of rural women? How can those be addressed by different stakeholders?
What are good practice strategies for gender-responsive rural development poverty and hunger eradication and food and nutrition security?
What changes do we need to empower women smallholders and achieve food security?

Concluding remarks and recommendations

The majority of the rural poor are small holder farmers, who are responsible for most global food productions. Helping them to improve farm productivity through better access, to resources, technologies, markets and organizations will be critical for both poverty eradication and food security. Small holder farmers will need affordable access to technologies, and infrastructure to sustainability transform food systems.
Poverty cannot be eradicated without addressing the pervasive inequalities in incomes and economic opportunities between and within countries, between rural and urban areas and between men and women. Reducing such inequalities will need to start with improving access for the poor to productive resources, basic services and social protection.
Greater access to information, organizations, and resources is important for poor women, who disproportionately lack access compared for their men access compared for their men counter parts. Capacity building is needed for poor women in particular, as cultural and other gender-specific constraints have hindered them from greater engagement in market.
Having access to food of sufficient quality does not automatically translate into good nutritional status for individuals. Women's role in food utilization for food security is perhaps the most critical and outweighs the importance of their role in food productions and how they spend the income they earn.
There is now a growing recognition in policy that women's role is essential to food and nutrition security. Such policies tend to focus on increasing women's productive and economic capacity. This only a partial solution, failing to address the entrenched gender inequalities that prevent many women and girls from living a fulfilled life, free from hunger, poverty, and discrimination.
The precarious state of hunger and malnutrition in the world and the disproportionate in the world and the disproportionate impacts on women and girls highlight a conundrum. There is sufficient evidence to show why people are hungry and malnourished, but existing approaches- in terms of policy and practice- are failing to make headway in bringing about the substantive changes needed to ensure no one goes hungry.
Ensuring food security is both the most basic of development issues and the most complex. Gender inequalities are a significant part of problem and reducing those inequalities will be critical part of the solution. The inequalities women as producers reduce the potential productivity of agriculture and hence of overall food availability in countries, regions and worldwide. Failing to overcome the inequalities most rural women are facing; the crucial issues concerning eradication of poverty, fighting hunger, reaching food security and tackling food crisis will totally evaporate.

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PRODUCTION CAPACITY IN RURAL AREA OF ŽABLJAK MUNICIPALITY, MONTENEGRO

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Abstract

The capacity production in rural area was estimated at 16 farms holdings in the municipality of Žabljak. Within these estimated farm holdings are 3 cattle breeding and 13 combined direction of production. Among investigated farms were established differences for number of livestock, arable lands, number of employed member on family farm. The livestock number was different, depending on type of farms. In average on 10 ha of arable land, on cattle breeding farms there were 9 livestock, while on farms with combined direction of production there were 11.46 livestock. These farm holdings deal exclusively with organic-ecological production and are mainly focused on livestock production, with a dominant breeding of sheep and cattle. In average 4.7 members per farm holdings on cattle breeding farms and 5.3 members on combined farms were registered. In the rural area of Žabljak municipality the number of employed people per farm holding is lower than average value in Montenegro. Production is partly intended for the market because one part of the product reserves is for their own needs. In the municipality of Žabljak the quantity of production for market is approximately equal for nutrition. In average on both type of farm holdings, the share of own cost (80.8%), purchase (15.3%) and services (4.0%) is in structure of total production cost. The income of production per 1 ha of arable land was higher on cattle breeding (500 euros) than on combined, while income per one full time employee (4129 euros) on combined farm holdings as well per one family member (1675 euros) was higher than on cattle breeding farm holdings (2897 euros and 1241 euros) in this municipality.

Keywords: *rural area, production, farms, Žabljak.*

Introduction

The geographic, climatic and biological characteristics of the municipality of Žabljak is in accordance with the most stringent criteria for the production of pure organic food, organic agriculture and of very high quality (The Municipal Assembly of Žabljak, 2012). In the villages of municipality of Žabljak, the main activity of the population is livestock production. The importance of animal production is great, considering that livestock breeding and the production of animal products are the main sources of income from the agricultural production of farm holdings (Bajcetic, 1982; Bastajic, 2004). That means that the current existence of individual agricultural producers, as well as increasing income and commodity exchange of these farm holdings are primarily related to increasing livestock production (regardless of racial composition of livestock and the degree of intensification of production) (Bulatovic, 2001; Ceranic et al. 2009). However, the lack of livestock feed, as a factor in the function of livestock production, is not restricted because of small area of existing farmland (Bastajic, 2002; Bastajic, 2006). The main cause is an irrational and disorganized use of existing sources of livestock feed, especially natural meadows and pastures as a primary source livestock feed (Krstic, et al., 2003). The existing climatic conditions and existing agricultural capacity and subjects can provide a greater volume of production, but only a

certain quality and structure. Natural factors, particularly in the mountain area, of this region, have influence to management system and narrowed the structure of production in agriculture, which led to specificity of agricultural production (Birovljev&Tomic, 2001; Sredojevic, 2006).

The aim of this paper is the analysis of the productive capacity in rural areas of the municipality of Žabljak, based on estimation capacity of farms holdings of cattle breeding and combined direction of production, connected to size of farms, production cost, purchase and services, employment of the members of family and income per unit of land and per farm.

Material and method

Analysis of organizational and economic conditions (family members, land, livestock, and structure of production) and the results of the final production (income, value of the final production, and structure of production costs) of individual farms in rural area of municipality of Žabljak can indicate trends of interest to the extent of agricultural policy in this area. In this paper it was conducted the analysis of production for 16 farms of the municipality of Žabljak. For obtaining data it was used a questionnaire in a year-round survey in 2014. The survey included an above-average size of farm holdings, which are focused on a market-oriented production.

Results and Discussion

Organizational and economic conditions

The investigation included farms with large families in municipality of Žabljak. Combined direction (cattle breeding and sheep breeding) of production is related to the family with a large number of members, which requires the continuous involvement of at least one family member. For the researched farm holdings are characterized at least two family members permanently employed in their own possession, that approximately at every second household comes per one family member employed full-time, and that only one person works abroad of hundreds of work-age family members (table 1).

Table 1. Family members and the labor force on the farms in the municipality of Žabljak

Direction of production	Number of farm holdings	Total members per farm holdings	Work on the farm			Total of employes on farm holdings	
			Full time	Part time	Seasonal	In country	Abroad
Cattle breeding	3	4.7	2	1	1.3	2	-
Combined	13	5.3	2.2	1.5	0.8	5	-
Total	16	5.2	2.1	1.4	0.9	7	-

Source: Author’s elaboration based on the questionnaire survey results.

In Žabljak municipality there are in average 12 ha of arable land per farm holdings, which is approximately to maximum of land. Households with a combined direction of production have over 1 hectare larger surface area than farms with cattle production direction. All studied farm holdings use land under the annuity. In this regard stand out to farm holdings with a combined direction of production in Žabljak municipality use under the lease in average 6.19 ha (table 2). The land under lease in relation to family members, in average is 3.57 ha, that exceeds the national average. Earlier studies have shown that the category of farm households with an arable land over 10 ha, mostly participate in using of land under lease, which is connected to the number of working-age family members. In Žabljak municipality, the relationship between the land area and members of family is more favorable on farm holdings with combined direction of production than on farms with cattle breeding, as the only branch of animal husbandry. On this phenomenon, in addition to the direction of production, other

factors have influence including significant regional specificities and the possibility of using land in social ownership that is not involved in an organized production (Bastajic, 2006; Krastic et al., 2003).

Table 2. Average ownership of land per farm holdings of municipality of Žabljak

Direction of production	Number of farm holdings	Available arable land				
		Own land (ha)	Under lease		Total (ha)	Per family member
			ha	%		
Cattle breeding	3	11.04	0.53	4.58	11.57	2.48
Combined	13	12.80	6.19	32.59	18.99	3.57
Total	16	12.47	5.13	29.14	17.60	3.39

Source: Author’s elaboration based on the questionnaire survey results.

Analysed farm holdings possess on average 11 livestock units or more than one livestock units per 10 ha of arable land. Definitely the proportions between livestock and arable land on the mountain farm holdings would not be possible in our conditions in the non-use of socially owned land in feeding (pasture) cattle (Ceranic et al., 2009). This finding goes in favour of the fact that farm holdings with a combined direction of production absolutely and relatively have greater representation of livestock from other farm holdings, because precisely sheep breeding is more closely involved in the use of our own and community pastures (table 3).

Table 3. Average number of livestock at the end of 2014 in farms of the municipality of Žabljak

Direction of production	Number of farm holdings	Cattle		Sheep		Livestock number on 10 ha of arable land
		Total	Cows	Total	Breeding sheep	
Cattle breeding	3	27	14	-	-	9.00
Combined	13	177	83	615	549	11.46
Total	16	204	97	615	549	11.15

Source: Author’s elaboration based on the questionnaire survey results.

The structure of final production is in line with the direction of production. For farm holdings with the cattle direction of production, this branch participates with 60% in the final production, which is characteristic of highly specialized farms. Although, farm holdings with a combined direction of production are focused on livestock production and on this criterion they have the characteristic of specialized holdings, but their production is diversified. The representation of sheep breeding with (30%) in the structure of the final production on these farm holdings has resulted in reduction of crop production. Analysed farms are equal by the structure of the product realization (table 4).

Table 4. The structure of the final production of agriculture in farms of the municipality of Žabljak

Direction of production	Number of farm holdings	Field		Fruit		Cattle breeding		Sheep breeding		Other		Total	
		€	%	€	%	€	%	€	%	€	%	€	%
Cattle breeding	3	5801	26.2	308	1.4	13295	60.1	-	-	2724	12.3	22130	100
Combined	13	21297	13.8	6014	3.9	70185	45.5	47421	30.7	9399	6.1	154318	100
Total	16	27099	15.4	6322	3.6	83481	47.3	47421	26.8	12094	6.8	176448	100

Source: Author’s elaboration based on the questionnaire survey results.

The final production in farms of the municipality of Žabljak

The market participates in the structure of realization of the final production from 3/4, which illustrates the market orientation of these farm holdings. The high share of commodity production in the total production is related to the nature of livestock production (table 5).

Table 5. Values of agricultural production in farms of municipality Žabljak

Direction of production	Number of farm holdings	Final production					
		For the nutrition		For the market		Total	
		€	%	€	%	€	%
Cattle breeding	3	6276	28.4	15854	71.6	22130	100
Combined	13	40270	26.1	114047	73.9	154317	100
Total	16	46546	26.4	129901	73.6	176447	100

Source: Author's elaboration based on the questionnaire survey results.

For the analysis of the intensity of production on farms are used data related to the cost of materials and services. Individual production participates in the material costs of reproduction with 80%, which is a significant feature of the reproduction of farm holdings in the mountain region. Households with a combined direction of production show a lower share of individual production in the structure of the material costs (table 6), which can be explained by the nature of the technological process in the sheep breeding (Bastajic, 2006).

Table 6. The structure of production costs in farms of the municipality of Žabljak

Direction of production	Production costs								
	Own		Purchase		Services		Total		
	€	%	€	%	€	%	€	%	€ / ha
Cattle breeding	16470	83.2	1880	9.5	1440	7.3	19790	100.0	56983
Combined	117690	80.4	23470	16.0	5150	3.5	146310	100.0	59259
Total	134160	80.8	25350	15.3	6590	4.0	166100	100.0	59978

Source: Author's elaboration based on the questionnaire survey results.

The value of final agricultural production per farm holdings is higher in the direction of the combined production than in the cattle breeding, which can be associated with the number of family members and the larger capacity of the land in farm holdings with a combined direction of production. Direction of production equalizes more or less final production per household regardless of the geographical location of the farms. Reducing the final production by a unit of arable land, by the employee on the farm and by the family member incurred significant differences (Ceranic, et al., 2009).

Table 7. The value of the final (net) production (in euros) in farms of the municipality of Žabljak

Direction of production	Total per farm	By 1 ha of arable land	By one full time employee	By one family member
Cattle breeding	7376	637	3688	1580
Combined	11870	625	5511	2236
Total	11028	626	5189	2125

Source: Author's elaboration based on the questionnaire survey results.

The value of the final production per 1 ha of arable land was approximately equal (in euros) in both, farms with the combined direction of production and farms of cattle breeding, in the municipality of Žabljak (table 7). In farms with the combined direction of production, with permanently one employee as well in case with employee a family member of owner of farm holdings, the final production (in euros) was higher than in farms of cattle breeding in municipality of Žabljak. Earlier studies (Bajcetic et al., 1982) showed that the highest value of the final production per unit of arable land are achieved by producers of vegetables in suburban areas, followed by the producers of eggs, tobacco and grape producers.

Table 8. Income from agricultural production in farms of the municipality of Žabljak

Direction of production	Total €	By 1 ha of arable land	By one full time employee	By one family member
Cattle breeding	5795	500	2897	1241
Combined	8894	468	4129	1675
Total	8313	472	3912	1602

Source: Author's elaboration based on the questionnaire survey results.

Significantly higher total income from agricultural production is realized by farm holdings with the combined direction of production (table 8). Households with the direction of cattle production from the municipality of Žabljak have less average income.

Conclusion

Based on the analyzed rural households in the municipality of Žabljak it may be concluded that there is a dominance of farms of combined direction of production. These farms employ a larger number of family members than farms with cattle direction of production. Also, farms with a combined direction of production with significant capacity of the land, as land in its ownership, and by the participation of the land are leased. Livestock is the backbone of agricultural production in rural households with combined direction of production (the share of cattle breeding and sheep breeding accounts for about 75% of total production).

Both types of farms (cattle and combined direction) have approximately the same results in terms of the realization of the final production, with a slightly more favorable valuation code have farms with a combined direction of production. Also, households with combined direction of production in the municipality of Žabljak show a lower share of own production in the structure of the material costs, which can be explained by the nature of the technological process in the sheep breeding.

The income of production per 1 ha of arable land was higher on cattle breeding (500 euros) than on combined, while income per one full time employee (4129 euros) on combined farm holdings as well per one family member (1675 euros) was higher than on cattle breeding farm holdings (2897 euros and 1241 euros) in this municipality. All this indicates that rural households with combined direction of production in the municipality of Žabljak have significant advantages to be exploited, both in terms of the organizational, and in terms of production characteristics.

However, it should be emphasized that the current utilization of agricultural capacities and potentials in municipality of Žabljak is very poor, which faces the material base of the rational employment of numerous agricultural populations in this region, which has a negative impact on the income and overall living conditions. Also, sporadic local experience, show that agriculture of mountain regions can achieve fairly good results.

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PERISHABILITY AND POST-HARVEST MANAGEMENT OF SPICES IN IBADAN, NIGERIA

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Abstract

During the post-harvest period, spice crops are subject to losses due to a variety of causes-chemical changes, insect damage, rodent attack, and growth of microorganisms. It therefore becomes imperative to examine the perishability and postharvest management of spices (indigenous and exotic) in Ibadan, Oyo State, Nigeria. A well-structured questionnaire was used to gather information from fifty (50) randomly selected farmers in Lagelu local government area of Ibadan, Oyo State. Data were analysed using descriptive statistics and regression analysis. The study revealed that more men (90%) than women were involved in spices production and 50% were in the age group "41-60 yrs" while 62% had an average household size of 6-10 people. Sixty-two percent of the farmers stored their spices in baskets, 34% used bags and 4% spread out their spices in the open space. The drying method mostly used was sun drying (90%), 8% air dried their produce and 2% oven dried. On the average, 48.67% of the spices were non-perishable, 19.78% were mildly perishable and 31.56% formed the highly perishable spices. The regression results revealed household size, initial capital outlay and location of farm as socio-economic variables affecting income generated on spice production. It is therefore worthy of note that the farmers perspective of spices in the study area is that they are mostly non-perishable.

Keywords*: Spices, Perishability, Post-harvest, Regression.*

Introduction

Spices are aromatic and pungent herbal substances used as condiments. These substances generally give a characteristic smack, flavor, color and aroma (Khan and Balick, 2001; Gurib-Fakim, 2006). In Nigeria, spices act as food buffer and create a source of income for the farmers and marketers. They are also known to possess medicinal and nutritional uses ranging from treatment of cough, pneumonia, appetite boosters, womb cleansing and several others (Sethberger *et al*, 1996).

Spice crops can be classified into various categories based on their degree of perishability: non-perishable, semi-perishable and perishable spices (Atibudhi, 2009). Studies indicated that post-harvest losses in non-perishable ranged between 10-15 percent; loss in semi-perishable crops, 15-30 percent and that of perishables 25-40 percent (Hussain, 1993). Estimates of postharvest losses in developing countries vary greatly from 1% to 50% or even higher (FAO, 1981). During the post-harvest period, spice crops are subject to losses due to a variety of causes: chemical changes, insect damage, rodent attack, and growth of microorganisms. By far the greatest losses occur through growth of microorganisms.

Different types of preservation principles such as application of heat (blanching, pasteurization, sterilization), removal of heat (cooling/freezing.), drying and dehydration, use of different chemicals and preservatives, irradiation and controlled/modified atmospheric storage have been practiced for extending the shelf life of perishable commodities. However, till date the most commonly adopted method for long-term preservation is the removal of

moisture due to less cost of operation with acceptable quality. The dehydrated foods have gained market acceptance because of their economic viability, higher degree of inhibition of microbial attack, lower cost involved in transportation, handling and storage, and minimum labour requirement for production and packaging (Atibudhi, 2009). A good amount of markets in the country and abroad can also be built up for dehydrated indigenous spices, which are known for their exotic flavour and other qualities.

The drying process for most of the spices produced in the country is usually either under sun or by using conventional dryers. However, most of these spices have a limited post-harvest shelf life due to mechanical, physiological and pathological factors. Post-harvest technologies which greatly influence the level of post-harvest losses include, grading, packaging, transportation and storage.

It therefore becomes important to examine the perishability and post-harvest management of these spices with the following objectives; to determine the socio-economic characteristics of the respondents, to examine the farmers' perspective of spices perishablilty along-side methods adopted in managing post-harvest losses amongst farmers in the study area and to identify the factors affecting income generated from spices production in Ibadan, Oyo State.

Material and Methods

Ibadan, the capital of Oyo State, is the largest city in Nigeria. It is located in the South-Western part of the Country. There are 11 local government areas (LGAs) in Ibadan and its environs.

Lagelu local government area in the metropolis of Ibadan was purposively selected for the study on the basis of concentration of spice farmers (though still meager compared to other food crops) in Ibadan, Oyo state. Five (5) villages; Edun, Lalupon, Apatere, Lagun and Iyana-offa were purposively selected on the basis of their production of spices in the L.G.A. Primary data were collected from a sample of fifty (50) farmers (10 farmers from each village) through random sampling technique.

Well-structured questionnaires and interviews formed the means of data collection. Data collected were analysed using descriptive statistics, regression analysis and ranking. The perspective on perishability were measured using the 3-point scale as non-perishable =1, mildly perishable = 2 and highly perishable = 3.

Also, the linear regression model used was specified in the general form as:

$Y = f(x_1, x_2, x_3, x_4, x_5)$

Where:

Y = Income from spices in ₦/ bag
X_1 = Age (Years)
X_2 = Household size
X_3 = Educational Level
X_4 = Initial Capital Outlay
X_5 = Location of spices

Results and Discussion

Socio-economic Characteristics of Respondents

The result of the analysis in table 1 showed more male respondents (90%) than females. Half of the respondents were in the age group 41-60 yrs showing most of the respondents as matured and elderly people. About 96% of the respondents were married, 66% were Muslims depicting the dominance of that religious sect in the study area.

Furthermore, 44% of the respondents were primary school certificate holders which formed the highest proportion of the respondents. More than half of the respondents (62%) had an

average household size of 6-10 people. The predominant ethnic group was the Yoruba tribe (100%) while 84% of the respondents took up farming as a major occupation. The highest average monthly income of the respondents which is in the range ₦0-50,000 was 68% showing most of the respondents as small-average income earners. Also, the highest years of experience in farming (11-25yrs) was 30% which invariably suggests that some of the respondents were well experienced in the cultivation of spices.

Table 1. Socio-economic characteristics of the respondents

Variable	Frequency	Percentage
Sex		
Male	45	90
Female	5	10
Age		
<=25	1	2.0
26-40	1	2.0
41-60	25	50.0
61-80	23	46.0
Marital status		
Married	48	96.0
Divorced	1	2.0
Widowed	1	2.0
Religion		
Christianity	14	28.0
Islam	33	66.0
Traditionalist	3	6.0
Education		
Primary	22	44.0
Secondary	9	18.0
Tertiary	3	6.0
Illiterate	16	32.0
Household Size		
2-5	7	14.0
6-10	31	62.0
11-16	10	20
17-23	2	4.0
Ethnicity		
Yoruba	50	100
Occupation		
Farming	42	84.0
Salary earner	1	2.0
Farming & Others	7	14.0
Average income from spices		
₦ 0 – 50,000	34	68.0
₦ 51,000 – 200,000	11	22.0
₦ 201,000 – 500,000	3	6.0
₦ 501,000 – 1,000000	2	4.0
Years of Experience		
4 – 10	11	22.0
11 – 25	15	30.0
26 – 45	13	26.0
46 – 70	11	22.0

Source: Field survey 2012

Management of Post-harvest Losses

As shown in Figures 1 and 2 below, the methods commonly used in storing, preserving and drying spices generally among the respondents are sun-drying, air drying and oven drying locally, while storage is done in bags, baskets and spread out in the open space for air. Sixty-two percent of the farmers stored their spices in baskets, thirty – four percent used bags and four percent spread out their spices in the open space for proper aeration.

The drying method mostly used by the farmers (90%) was sun drying, while about 8% air dried their produce and 2% oven dried. Atibudhi (2009) in his study also highlighted sun-drying as the most prominent drying method used for spices and other food crops. This could be due to the cost effectiveness of sun drying method over other methods.

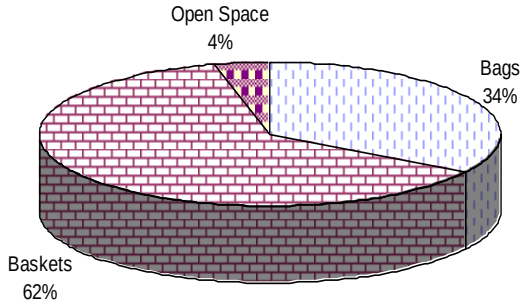

Fig. 1: Storage Medium for Spices

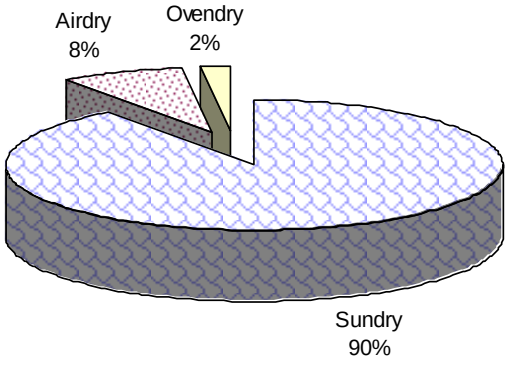

Fig. 2: Drying Methods for Spices

Perspective on Perishability of Spices

Nine spices (4 exotic & 5 indigenous) were sampled from the farmers. Data on the percentage shelf-life (high, moderate and low) of these spices were captured according to farmers' perspective and analysed using frequency distribution.

From the analysis in Table 2, *Tetrapleura tetraptera* and turmeric tied (88%) as the highest percentage of non-perishable spices. Also, garlic and *Parkia biglobossa* tied (38%) as the highest percentage mildly perishable spices. Ginger (80%) was singled out as the spice with highly perishable properties which could be as result of the high moisture content it carries. This supports the study by Atibudhi (2009) who buttressed that senescence takes place in ginger due to biochemical changes and high water content, which results in loss of production.

On the average, 48.67% of the spices had a high shelf-life, 19.78% had a moderate shelf-life and 31.56% averagely formed the low shelf-life category of spices. It is therefore worthy of note that the farmers perspective of spices in the study area is that on the average they possess a high shelf life.

Table 2. Respondents' Perspective on Shelf-life of Spices

SPICES	HIGH SHELF-LIFE (%)	MODERATE SHELF-LIFE (%)	LOW SHELF-LIFE (%)
Garlic	12.0	38.0	50.0
Black pepper	66.0	12.0	22.0
Hot pepper	6.0	34.0	60.0
Ginger	6.0	14.0	80.0
Xylopia	78.0	2.0	20.0
Efirin	42.0	28.0	30.0
Tetrapleura	88.0	6.0	6.0
Parkia	52.0	38.0	10.0
Turmeric	88.0	6.0	6.0
Average shelf-life (%)	**48.67**	**19.78**	**31.56**

Source: Field survey 2012

Factors Affecting Income Generated On Spice Production

The regression results in table 3; revealed household size, initial capital outlay and location of farm as socio-economic variables affecting income generated on spice production. This suggests that the low household size of the respondents could lead to the meager income generation from the production of spices in the study area, if their major source of labour is the farm family. Also, the initial capital outlay which was significant at $P<0.05$ showed that available capital invested in any production process vi-a-viz spices production will either enhance or minimize profitability. The significance of the location of the farm ($P<0.05$) however showed that, locational differences (weather conditions, pest and disease, accessibility to farm inputs amongst others) which exist in agricultural production might lead to an increase or a decrease in income generated. The socio-economic variables; such as age and educational level; did not affect income generated on spices production in Ibadan.

Table 3: Result of linear regression analysis

Variable	Coefficients	Standard Error	Y-Value
Constant			0.867
Age	-0.128	0.151	-1.050
Household-Size	0.329	0.136	2.715*
Educational level	0.178	0.047	1.481
Initial Capital Outlay	0.247	0.105	2.007**
Location of farm	-0.495	0.066	-4.025**
R Squared = 0.460	Adjusted R^2 = 0.370	**Significant at 5%	*Significant at 10%

Source: Field survey 2012

Conclusion

Spices production in the study area is male dominated with elderly people as farmers.
Farming is the major occupation of the respondents and a meager income of ₦ 0 – 50,000 per month is realized by most of the farmers who have a production experience of 11-25 years the most. On the average, 48.67% of the spices had high shelf-life, 19.78% had moderate shelf-life and 31.56% averagely formed the spices with low shelf-life.

It is however worthy of note that the perception of these spices by the farmers in the study area is that on the average they possess a high shelf life. It is also recommended that more awareness on the medicinal, nutritional and economic importance of spices be reiterated so as to enhance production, improve the shelf-life of spices and create a balance between demand and supply of spices in Nigeria.

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REMITTANCES, POVERTY AND INCOME INEQUALITY IN RURAL PAKISTAN: LINKAGES AND EMPIRICAL EVIDENCE

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Abstract

This paper analyses income inequalities in rural Pakistan. Using micro data (Survey 2008) allows us to decompose income inequality according to its different sources. Our dataset comprises all four provinces of Pakistan. To ensure representativeness, we decided to sample households in 10 districts (i.e. roughly one tenth of the total number of districts) across the country (stratified sampling). The number of districts has been selected in proportion with the size and population of each province. Two villages were chosen from each district. Within these predefined strata, households have been selected randomly. Our target was to achieve a total of 30 responses per village, that is 60 house-holds per district, yielding an overall sample size of N=600. Different variables are used in the paper: Monthly income of the household decomposed into five major income sources such as transfer income, agricultural income, non-farm income, rental income and livestock income. We calculate Gini coefficients and Theil indices both within and across the provinces and districts. We find that in-equality between districts is higher than within districts. A partial correlation analysis extends our descriptive investigation to reveal the different impacts of the various income sources on overall income inequalities. We observed that non-farm and transfer income have the strongest impact on income inequality across districts both in economic and econometric terms. While the former source of income is increasing inequality, transfer income tends to reduce in-equalities. Our analysis suggests important policy implications. It emphasises in particular the need for factor mobility to facilitate transfer income.

Keywords: Income inequality; poverty; remittances; economic development; rural Pakistan.

Introduction

All over the developing world policymakers are interested in devising new strategies for rebalancing skewed income distributions and reducing poverty. The choice of such strategies crucially hinges on an improved understanding of the sources of income inequality. Why do certain types of incomes go to particular groups of people? And what roles do variables such as land-ownership, migration and education play in improving income distribution and in lifting people out of poverty? Using primary household survey data, we identify different types of income to disentangle each source's impact on overall income inequality in Pakistan's four provinces. Our dataset is wide-ranging, providing rather detailed information on different districts across the country.

Other authors such as Adams and Alderman (1992) and Glewwe (1986) use panel data which covers up to three years, but are less detailed on the household level. We resort to a cross-sectional, yet well-designed, framework. Similarly, Adams (1994) uses three year panel data to analyse the impact of non-farm income on income inequality in rural Pakistan. He found that non-farm income represents an inequality-decreasing source of income. Ahmad (2000) found that moving from household-level to individual data leads to a further improvement in the distribution of income. Ahmad (2002) finds that within the given occupational groups in the four provinces of Pakistan, the highest level of inequality is observed among skilled workers (Gini coefficient of 0.299), followed by inequalities amongst the group of

legislators/officials (Gini coefficient of 0.273). In contrast to that, the Gini coefficient among unskilled workers is 0.180 and is the lowest within the professional class (Gini coefficient of only 0.136). This may have been due to the fact that many of them were government employees and the wage structure was more equal in the government sector. Anwar (2005) found that the wage incomes being more unevenly distributed than in rural areas. Moreover, income from self-employment was more deviated in urban areas than in rural areas as urban self-employment ranged from wealthy businessmen to poor workers, whereas the bulk of the rural self-employed were a rather homogeneous group being mostly employed in informal sector enterprises. Nugent and Walther (1982) find that periods of bad weather crowd out workers from the low-income group and increase inequality. Good weather, on the other hand, opens up more job opportunities for the workers from the low-income group and hence reduces imbalances in the income distribution.

Glewwe (1986) observed that labor income inequality accounts for slightly more than half of overall inequality in urban and estate sectors but not in the rural sector, where total inequality attributes largely to non-labor income and income from the sale of agricultural products (accounted for as profits). Adams and Alderman (1992) found that imbalances in land ownership are not the main drivers behind inequality in agricultural income. Instead, income from returns to labor and crop profits contributes the most. Anwar et al. (2004) suggest that unequal landownership is one of the most important causes of poverty and inequality in rural Pakistan as land is the principal asset in an agrarian economy.

Materials and methods

The total sample size 600 was used. There is random selection of 60 households within 10 predetermined districts across Pakistan’s provinces (stratified sampling). We assigned weights: Punjab (= 0.4), Sind (= 0.3), NWFP (= 0.2), Baluchistan (= 0.1). A detailed questionnaire presented a differentiated picture in terms of income sources and household characteristics. Our main limitation is the static perspective (i.e. we do not consider changes over time).

Income sources

The individual households’ total income may stem from up to five sources, y_i:

Transfer income (y_t) includes income earned from migration, both within (from rural to urban sector) and outside (abroad) of Pakistan. Income earned from the first kind of migration is treated as internal remittances; income from the latter as external remittances.

Agricultural income (y_a) includes net income (cash as well as in kind) from all crop production plus wage earnings from agricultural labour. Nonfarm income (y_{nf}) includes wage earnings from nonfarm labour including self-employment, government and private-sector employment. Rental income (y_r) includes rents received from ownership of assets including land, machinery (tractors, threshers), buildings, and water. Livestock income (y_l) includes net returns from traded livestock (cattle, poultry) plus imputed values of home-consumed livestock. The overall monthly income shares of the sample households are given as follows:

Table 1**:** Monthly income shares. N = 600 households.

Source: Survey 2008.

y_i	S_i
y_{nf}	18%
y_a	23%
y_t	30%
y_l	15%
y_r	14%

Distribution of sample households by income quintiles

The distribution of the sample households by income quintiles is shown in Table 2. Two points are worthwhile noticing: First, the shares of transfer, agricultural and livestock income are relatively higher in lower income quintiles as compared to the higher income quintiles and may be considered as major sources of income for the households belonging to lower income quintiles. In contrast to that, the shares of rental and nonfarm income tend to be higher in higher income quintiles. Second, the share of livestock income is lowest in the monthly income of the households reported in the top income quintile, whereas the share of rental income is lowest in the bottom quintile. Overall, we may say that the distribution is actually very similar for the bottom 4 quintiles and the differences really come with the richest quintile.

Table 2: Distribution of sample households by income quintiles.
Source: Survey 2008.
Note: All income shares are expressed in percentages.

Sample Distribution (%)	Income Groups (in PKR)	S_{nf}	S_t	S_a	S_r	S_l
Bottom quintile	1596-2526	16.4	31.9	23.9	9.0	18.8
2nd quintile	2550-3116	16.4	30.6	23.2	10.2	19.6
3rd quintile	3136-3359	13.2	33.1	23.6	10.3	19.8
4rth quintile	3365-4298	16.6	30.8	22.3	13.5	16.8
Top quintile	4371-6938	22.8	27.1	21.7	21.2	7.2

We can identify the impact of each of the different sources of income on overall income inequality as follows:

• breaking-down household's total income in the $n = 5$ sources:

$$y = \sum_{i=1}^{n=5} y_i,$$

• calculating weights of income source i:

$$w_i = \bar{y}_i / \bar{y},$$

where $\bar{y}_i$ represents the mean monthly income of source i and $\bar{y}$ is the mean of total monthly household income.

• calculating the correlation ratio between income source and total income:

$$R_i = \frac{cov(y_i, r)}{cov(y_i, r_i)}$$

where r is the ranking of total income such that higher income receives a higher rank and r_i expresses the corresponding ranking of income sources.

• determining the Gini coefficient associated with income source y_i:

$$G_i^2 = \frac{}{N \bar{y}_i} cov(y_i, r_i),$$

allows us to decompose the Gini coefficient for rural Pakistan $G_{rPk} = 0.189$[24] (Survey, 2008) to measure the contribution of the different income sources to overall income inequality c_i:[25]

Results and discussion

Income decompositions: Decomposing overall income inequality by income sources

Table 3, shows contribution to overall income inequality by each of the income sources as given below. According to the table, agricultural, nonfarm, rental and transfer income each contributes approximately one-fourth (i.e. 1/4) of the overall income inequality while livestock income hardly contributes to the total inequality.

Table 3: Contribution to overall income inequality by income source.

Source: Survey 2008.

y_i	w_i	R_i	G_i	c_i
y_a	0.23	0.99	0.17	0.21
y_l	0.15	0.13	0.16	0.02
y_{nf}	0.18	0.88	0.31	0.26
y_r	0.14	0.97	0.37	0.27
y_t	0.30	0.95	0.17	0.26

Inequality decomposition by regions: Decomposition of income inequality within and between provinces

Given the design of our survey, the sample is readily partitioned to differentiate between inequality within (I_w) and between (I_b) regions where it obviously holds that $I = I_b + I_w$.
Using this methodology we can first decompose the Theil Index on a more aggregate province level as follows:

$$I = \underbrace{\sum_{k=1}^{K=4} \frac{N_k}{N} \left(\frac{\bar{y}_k}{\bar{y}}\right) \ln\left(\frac{\bar{y}_k}{\bar{y}}\right)}_{Between} + \underbrace{\sum_{k=1}^{K=4} \left(\frac{N_k}{N} \frac{\bar{y}_k}{\bar{y}}\right) I_k}_{Within}$$
$$= 0.0016 + 0.0634 = 0.055$$

The results of above equation suggest that inequality on this level of aggregation is almost exclusively driven within rather than between the provinces.

Decomposition of income inequality between and within districts

[24] $\sum_{i=1}^{5} \text{wiRiGi} = 0.189$

[25] $c_i = w_i R_i \frac{G_i}{G_{rPk}}$

Unlike Gini coefficients, the Theil index has the property of additive decomposability. On the more disaggregated district level we have,

$$I = \underbrace{\sum_{j=1}^{J=10} \frac{N_j}{N}\left(\frac{\bar{y}_j}{\bar{y}}\right) ln\left(\frac{\bar{y}_j}{\bar{y}}\right)}_{Between} + \underbrace{\sum_{j=1}^{J=10}\left(\frac{N_j}{N}\frac{\bar{y}_j}{\bar{y}}\right) I_j}_{Within}$$
$$= 0.0535 + 0.0015 = 0.055,$$

$$R_b = \frac{I_b}{I} = \frac{0.0535}{0.0550} = 0.97.$$

which reveals that 97% of total inequality is explained by inequality between districts and inequality within the districts accounts for the remaining 3%.

Partial correlation analysis

In this section, we consider partial correlation coefficients to measure the statistical relationship between income inequality (G_j) and the shares of different income sources ($S_{i,j}$) across the districts, where all variables are expressed in terms of percentages and G_j represents the Gini coefficient as measure of overall income inequality in the j^{th} district and the share $S_{i,j}$ is defined as the ratio of average income of type i in district j over total average income in district j, as shown in Table 4, such that:

$$S_{a,j} = \frac{\bar{y}_{a,j}}{\bar{y}_j} \times 100$$
$$S_{t,j} = \frac{\bar{y}_{t,j}}{\bar{y}_j} \times 100$$
$$S_{nf,j} = \frac{\bar{y}_{nf,j}}{\bar{y}_j} \times 100$$
$$S_{r,j} = \frac{\bar{y}_{r,j}}{\bar{y}_j} \times 100$$
$$S_{l,j} = \frac{\bar{y}_{l,j}}{\bar{y}_j} \times 100$$

Table 4: Share of different income sources; by district.
Source: Survey 2008.
Note: All income shares are expressed in percentages.

District	Gini	Share of income source (%)				
	(%)	$S_{t,j}$	$S_{a,j}$	$S_{nf,j}$	$S_{r,j}$	$S_{l,j}$
Attock	5	27	22	26	19	6
Sahiwal	8	28	22	23	12	15
Layyah	5	29	23	20	10	18
Rahimyar Khan	5	30	23	18	10	18
Badin	2	33	23	17	10	17
Thatta	8	34	25	14	7	19
Mirpur khas	3	28	21	17	26	8
Dir	2	34	24	12	9	21
Malakand	4	29	22	13	16	20
Kalat	2	34	24	12	9	21

Table 5: Partial Correlation Analysis.

Note: *, ** and *** denote statistical significance at 10%, 5% and 1% levels respectively.

Partial Correlation of G_j with		
Variable	Corr.	Sig.
$S_{a,j}$	0.6494	0.114
$S_{t,j}$	-0.7354*	0.060
$S_{nf,j}$	0.7354*	0.060
$S_{r,j}$	0.6291	0.130
$S_{l,j}$	0.6517	0.113

Similar to a regression analysis, partial correlation seeks to measure a relationship between dependent and independent variable, whilst eliminating potential effects of a third variable. The partial correlation coefficients measure in this case the degree of statistical association between income source and district-wide Gini coefficient, where the latter one is considered as the dependent variable. Results are shown in Tables 5. Columns two and three report the partial correlation coefficient and the corresponding significance level, respectively. We find a strong positive and statistically significant correlation of 0.74 between the share of nonfarm income and income inequality across the districts. Thus, nonfarm income appears as inequality increasing source of income. In contrast to this, we detect a similarly strong (and also statistically significant) but negative correlation (-0.74) between the share of transfer income and income inequality. Unlike nonfarm income, transfer income is a source of income that is capable of reducing income inequalities in rural Pakistan. This result is in line with Shahbaz et al. (2014), exploring the dynamic linkages between income inequality, international remittances and economic growth using time series data over the period of 1976-2006, found that Pakistan is yet to benefit, in terms of reducing the gaps of income inequality, from the international flow of remittances and economic growth. Combes et al. (2014), using a cross country dataset (including both developing and underdeveloped countries), show that the level and the predictability of remittances and migration, reduce working poverty in receiving economies through their effects on labour market dynamics. Similarly, Beyene (2014), studies the effects of international remittances on poverty and inequality in Ethiopia using an urban household survey from 2004, finds a significant reduction in poverty, while inequality does not change with the inflow of international remittances.

Conclusion

We find that transfer income is the most important source of total monthly household income, accounting for almost 30% of its share. Shares of transfer, agricultural and livestock income are relatively higher in lower income groups as compared to higher income groups, shares of rental and nonfarm income tend to be higher in higher income groups. Agricultural, nonfarm, rental and transfer income each drive income inequality in the sample area to a similar degree. Livestock income hardly affects the results. Decomposing overall income inequality within and between districts reveals that overall inequality is largely determined by inequalities between (97%) rather than within districts. The partial correlation analysis confirms the

importance of transfer income in reducing income inequalities (negative estimated coefficient), in contrast, nonfarm income appears to increase inequality.
Policymakers should hence put more emphasis on transfer income in fighting in-equalities. For instance, they should try to foster its flows both between Pakistan and from the rest of the world. Particularly and initially try to increase factor mobility within the country by providing stronger technical education for the rural (and often poor) unskilled labour force so as to increase mobility and improve job opportunities within Pakistan as well as abroad. Furthermore, to raise the overall income level, policymakers should take steps to help poorer households send migrants abroad (the idea of "rural migration centers").

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COMMON AGRICULTURAL POLICY IN POLAND – ACHIEVEMENTS AND EXPECTATIONS DURING MEMBERSHIP IN THE EUROPEAN UNION

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Abstract

The agribusiness in Poland is still important and significant subsystem of the national economy. The analysis of factors influencing on structural changes in Polish economy demonstrates that the economic growth model prevailing in Poland is based on classical production factors: labour, land and capital. In the period 2004-2013 creation modern economy, macroeconomic conditions, globalisation and integration processes determined structural changes in agribusiness sector. In Poland was observed an absolute decline in the share of agriculture in the value added in the economy, employment, trade and consumption. Decreasing importance of agriculture is accompanied by an increase in income. The share of primary agricultural products in the value of finished products follows a downward trend, thus increasing the value added in the food industry. This process is already taking place, with the support of the agricultural policy activities. The purpose of this study is to assess the degree of development of agribusiness and its importance for the national economy and the presentation the achievement and expectations related with Poland's membership in the EU.

Keywords: *agribusiness, economic development, public support, competitiveness*

Introduction

The extent and type of instruments of the Common Agricultural Policy (CAP) of the European Union (EU) result from the postulate of an active role of government in the process of eliminating inefficiencies of market mechanisms within the concept of market failure (cf. Bator 1958, pp. 157-175). This concept suggests that in the conditions of the market economy, the structure and size of supply are not reflected in adequate level of demand as a result of the lack of autonomous re-balancing mechanisms (Medema 2007, p. 33). In practice, the processes of allocation of goods and services, despite the assumption of conditions of perfect competition and complete information as attributes of a complete market, show a number of difficulties (Hayek 1939). As a result, the state of actual equilibrium achieved by the market is characterised by the allocation of goods and services, which does not comply with Pareto optimum (Baumol 1952). In broader terms, the concept of market failure is a trend in economic theory which identifies the scope and circumstances of observed defects of market mechanisms that lead to the perpetuation of market imbalances. In this context, one points out the positive aspects of market intervention by public authorities (Stiglitz 1989).

The neoclassical model of structural changes in agriculture emphasises the relationships between the farm size and the scale and efficiency of production (Wigier, 2014). According to this model, it is only an appropriate scale of production that can ensure high efficiency (Chavas 2001). Therefore, economic growth and the physical size of farms can: improve their competitive position on the market, make it possible to use of economies of scale, result in reduced production costs, increase the bargaining power in trade, etc. The modern agricultural holding is characterised by a high degree of complexity, diversity and integration. Thus, the allocation of resources in agriculture becomes increasingly dependent on market forces and forming networks of interbranch connections. The agricultural production is increasingly dependent on the progress in genetic research, the implementation of advanced manufacturing

technologies, the development of research regarding the health and nutritional values of food, the application of organic production criteria.
The agrarian structure in Poland, which currently undergoes transformations, leads to an absolute reduction in the number of farms and polarisation of the area. Agriculture as a sector involved in the creation of the GDP loses its importance to the other sectors of the economy. Therefore, a characteristic feature of the process is deagrarianisation of the national economy and the development of rural areas. Throughout this process, it is extremely important for the ongoing structural changes to result in the improvement of the competitive position of farms and long-term and sustainable rural development. Poland's accession to the EU has generated new economic and organisational conditions to support structural changes in the broadly defined food economy and rural areas (Poczta 2012). Policy instruments implemented within the CAP create chances for the stabilisation of structural policy conditions over the period of several production cycles, thus stimulating the desired changes in the area structure of farms, the improvements in the competitiveness of production, environmental protection and multi-functional development of rural areas. Thus they are a fundamental instrument supporting the process of modernisation of Polish rural areas and agriculture (Łopaciuk et al., 2014).

Materials and methods

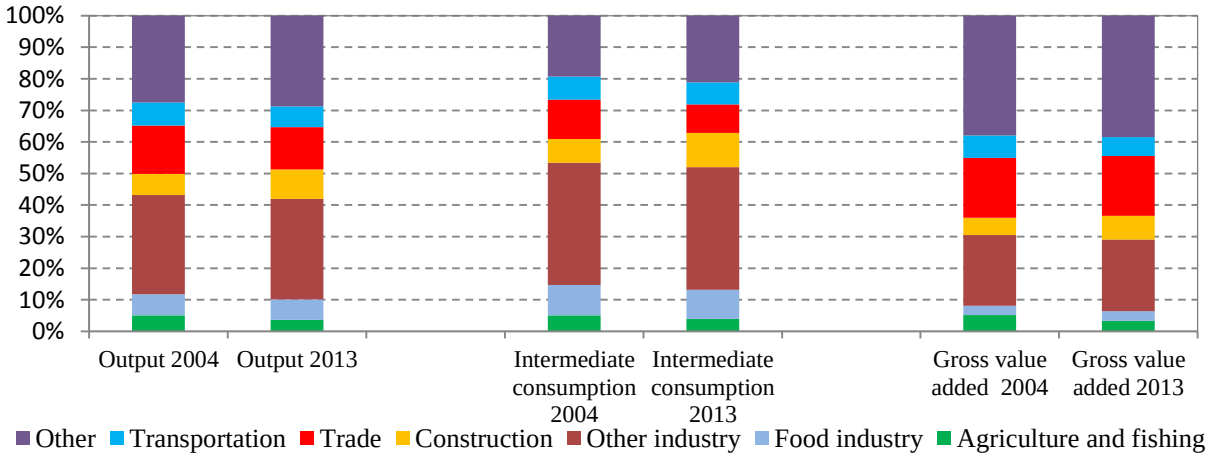
The purpose of this study is to analyse the development of agribusiness in the years 2004-2013, which includes an analysis of changes in its structure, the degree of integration of its components, the identification of reasons for changes taking place due to the European integration processes as well as the determination of strength and nature of the connections between the agribusiness sector and its environment. Using the statistical and descriptive methods, has been carried an analysis of the Central Statistical Office macroeconomic data with regard to assessing the degree of the development of agribusiness and its importance to the national economy. To study the impact of investments and public support on structural change in agriculture was used also one of the data mining methods - Grade Data Analysis (GDA). It is a family of algorithms and heuristics to extract knowledge from large data sets. GDA examines the relationship of objects and their properties, allowing for both verifying conjecture as to the specifics of an analysed phenomenon and identifying relationships not grasped by intuition on or even being in conflict with it. In results the GDA was a convenient tool for comprehensive data analysis.

Results and discussion

The current situation of agro-food sector in Poland is the result of the effect of multiple factors, associated with economic cycles, geopolitical ones and the current generation-related changes. Both macroeconomic conditions that arise from the presence within EU structures and the presence within the Single Market and State aid programmes addressed to the agri-food sector under the CAP contribute to it. The structural changes in agriculture result from the concentration of production and land. The inflow of EU funds from the EU was an important incentive that triggered structural changes and hence the improvement of the effectiveness of farming and the competitiveness of agriculture. However, the public policy instruments currently in use, which were supposed to promote convergence of the regions, are not able to prevent farm polarisation. Economic disparities increase between commercial farms with strong links to the market and the farms that produce mainly for self-supply and are social in their nature. The development distance between rich regions or the ones becoming richer and the poor regions clearly gets larger. Rich areas develop due to the use of their potential and economic situation whereas the poor areas are stuck in stagnation (Wigier, 2013).

Despite structural changes, sometimes very deep, the Polish agriculture remains an important sector of Polish economy. This is, primarily, confirmed by the structure of employment and structure of land use. The sector plays an especially important role as it comes to social and economic development of rural areas. Since agriculture uses over half of the total area of the country for economic purposes, it sets the main functions and directions of land use and shapes the natural environment and landscape. The agricultural sector remains the place of work for almost 15% of the total number of working people. However, the number of people working in agriculture points to negative relations between the labour resources and land and capital resources thereby causing low efficiency of labour. On the other hand, from the perspective of Gross Domestic Product (GDP) generation the significance of the agricultural sector in Poland is decreasing. Systematically was decreasing the share of agriculture in gross value added (from 5.1% in 2004 to 3.3% in 2013), and transport (respectively from 7.2% to 6.0%), whereas increased the share of construction (from 5.6% to 7.4%), services sector (from 37.9% to 38.5%) and industry (from 25.4% to 25.8%). The share of food industry in gross value added in the economy increased in this period by 0.1 percentage points (pp) to a level of about 3.1%. In the structure of the all industry, the share of the food sector was stable (approx. 11.8%) (Figure 1). The share of agriculture in replacement and increasing of the assets remains significantly smaller. Investment inputs for this purpose are shaped below 2%, which leads to further decrease in the role of agriculture as owner of fixed assets in the national economy.

Figure 1 The structure of outpu,
t, intermediate consumption and gross value added
of selected sectors



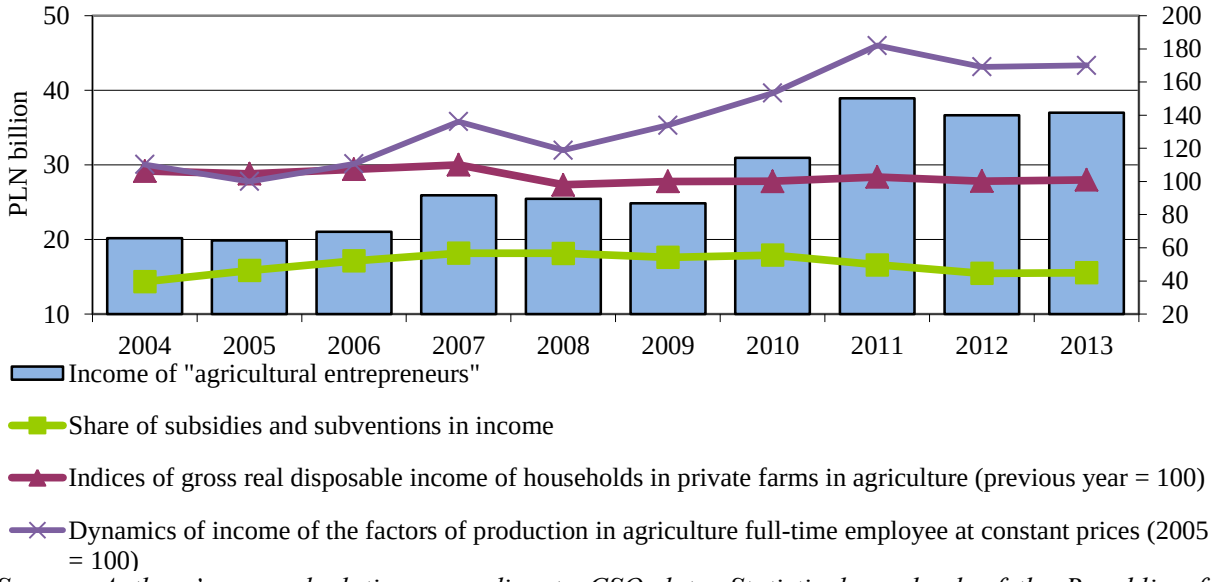
Source: Own calculations based on CSO data. Statistical yearbook of the Republic of Poland 2005 and 2014, www.stat.gov.pl, access date: 5 June 2015

There have been major changes in the agrarian structure which continued long-term trends. In the period preceding accession to the EU (1996-2002), large agricultural holdings (20-50 ha UAA), which took over arable land from small and medium agricultural holdings, but also from holdings of over 50 ha, developed dynamically. The growth in numbers was accompanied with the decrease of surface. The arable land of holdings below 1 ha grew, but their number dwindled. Following the accession to the EU, the number of holdings decreased by 28%, and their surface by 3%. The development of large holdings lost dynamics, but still arable land was taken over by holdings of the surface of 20 to 50 ha, for both smaller holdings, of which the number and area decreased, and bigger, the number of which, despite the decrease of the area, grew. The limiting of the number of the biggest holdings is relative to

the implementation of regulations limiting the area of family agricultural holdings and the ending of the lease period in second half of the previous decade. The number of holdings taking over land slightly increased. The number of holdings smaller than 1 ha decreased by 27%. To a slightly lesser extent the number of small and medium holdings decreased (from 25% to 16%). Their area also shrunk, including, to a largest extent, in the group of 10 to 20 ha (by 8%). These changes indicate that the Polish agriculture, despite major changes, is still to a large extent dispersed (Wigier, Darvasi 2012).

The income of the agricultural sector significantly increased after the accession to the EU. A sudden increase of income was noted in 2004 (over PLN (Polish zloty) 20 billion against less than 10 billion in 2002 and 2003), namely in the first year after Poland's accession to the EU and covering the national agriculture with the CAP income support system. In following years the dynamics of income growth was slowed down, yet a clear growing tendency was observed. In nominal prices their value in 2013 amounted to almost PLN 37 billion, as compared to less than PLN 10 billion in the pre-accession period (Figure 2). The growing income of the sector, combined with employment reduction, resulted in significant growth of income calculated per persons employed full time. In 2013 their amount was almost twice higher than in 2005 and almost twice as high compared with the pre-accession period. The share of subsidies and grants in income as a result of the implementation of direct payments and other measures investment aid schemes increased from 15 to more than 50 percent.

Figure 2. Income in the Polish agriculture and its dynamics in 2002-2013



Source: Authors' own calculation according to CSO data. Statistical yearbook of the Republic of Poland, www.stat.gov.pl, access date: 5 June 2015.

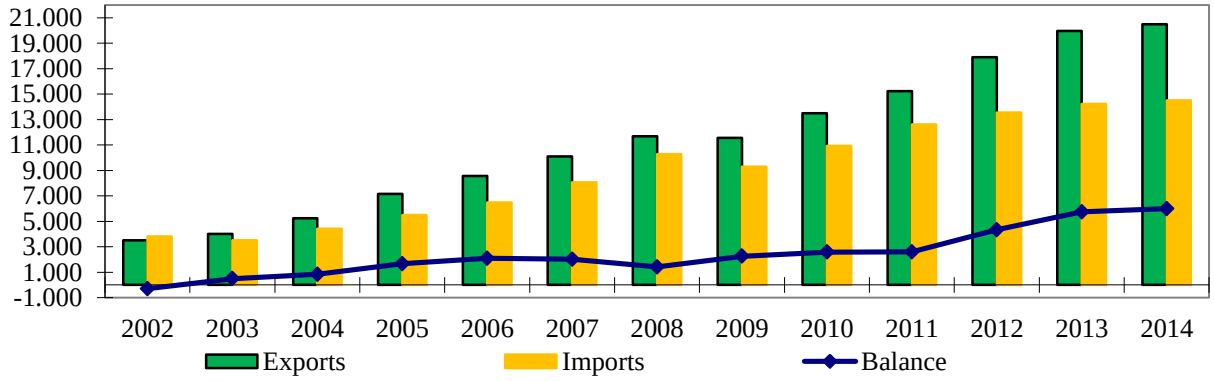
The integration with the EU created new conditions in Poland for the development of agriculture and food industry. Since 2002 the agro-food economy has been supported with the resources of programmes co-financed from the EU budget that penetrate and complement each other. These financial resources intended for agriculture development and paid from the EU budget may be divided into four groups according to their impact on growth and structural changes in agriculture:

entirely direct impact: modernisation of farms, early retirements and diversification of agricultural activity, setting up of young farmers;

entirely indirect impact: infrastructure, land drainage, land re-parcelling, afforestation, agri-environmental schemes, advisory services;

partly direct impact: direct payments, support for agricultural activity in less-favoured areas (LFA), market intervention expenditure, establishment of agricultural producer groups, establishment of micro-enterprises;
partly indirect impact: quality of life on rural areas, support to processing industry, PHARE programmes, LEADER programme, village renewal, training, technical assistance.
The EU funds had a significant share in the financing of transformations in agriculture until Poland's accession to the EU. The direct payments are the most common type of support; each year about 1.4 million of farmers use this form of support. The value of payments in the 2004-2013 period increased from ca. PLN 6 billion to PLN 14 billion per year. When calculated per one farm it reaches an average of ca. PLN 9 thousand, and this form of support is used by 87% of farms having an area of more than 1 ha. An equally important source of income (regardless of production, and only based on the farm's location) are payments for less-favoured areas (LFA). Each year these payments are granted to ca. 700 thousand farmers, i.e. half of those receiving direct payments. The land surface covered with LFA payments amounts to ca. 6.9 million ha. These payments are made to farmers on an annual basis. The manner of spending of the resources is not subject to settlement. Smaller farms usually allocate the granted payments to current needs and means of production (fuel, fertilisers), while the bigger ones also make investments. In the 2004-2013 period, the cumulative value of support for the agri-food sectors from three main sources of support, i.e. a grant from the national budget to Agricultural Social Insurance Fund , a grant from the national budget co-financing of the CAP, and payments from the EU budget, exceeded PLN 300 billion.
One of the noticeable effects of structural changes in the food sector is the agro-balance of foreign trade. Following years of stagnation or sluggish growth Polish foreign trade in agri-food products soared after EU accession. Previously a net importer of food products, Poland has become a net food exporter. In 2014 estimated export surplus amounted to about EUR 6 billion. The positive balance is generated by trade in products of the food industry, and the surplus is several times higher than deficit in agricultural trade (Figure 3). The share of the Polish agriculture in creating the added value following the accession to the EU shows a tendency to decrease. Currently it is at the level of 3-4%. At the same time, agriculture employs ca. 15% of the total number of employed people, which is indicative of low labour efficiency.

Figure 3 Polish foreign trade in agri-food products in 2004-2014 (EUR million)



Source: Authors' own calculation according to: Handel zagraniczny…

Conclusion

After the Polish accession to the EU, major changes took place in the formulation and implementation of the agricultural policy. They consisted mainly in systematising the policy objectives and increasing the expenditure earmarked for financing of changes in agriculture

and rural areas. The inclusion of agricultural holdings in the CAP mechanisms contributed to the improvement in the income situation of most farmers. The fundamental significance for the increase in the level of the income of farms was held by subsidies (mainly in form of direct subsidies). The improvement in the competitiveness of agriculture is, however, dependent on the transformations of a structural nature (which precondition the improvement in the efficiency of utilising the factors of production) and the development of the whole national economy, especially in the context of the capacity for the creation of new jobs outside agriculture, also in the rural areas.

The structural changes taking place in the Polish agriculture, food industry and rural areas in the last decade became more dynamic Within the last decade there has taken place the dynamisation of structural changes occurring in Polish agriculture. As the most important should be recognised: a decrease in the number of farms with a simultaneous increase in the share taken by the largest farms; the decrease in employment in agriculture and the progressing concentration and specialisation of production. The size of the investment has increased noticeably, but their value still does not exceed the value of depreciation of fixed assets. The investments were mainly in machinery and to a much lesser extent in buildings and structures. The impact of individual CAP instruments is different. It ranges from the greatest impact – that of direct payments – to the slight significance of programmes supporting semi-subsistence farms or structural pension having only a minimal coverage. Combination of direct payments and cross-compliance requirements causes that this form of support plays the key role in providing basic public goods through sustainable agricultural land management (maintaining environmental quality of the landscape, biodiversity, access to water, climate stability and air quality) or public goods not related to the environment (activity in rural areas).

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INNOVATION IN THE SECTOR OF FOOD AND BEVERAGE PRODUCTION ON THE POLISH EXAMPLE

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Abstract

Innovation is the basis of smart and sustainable economic growth through increasing production and distribution efficiency or introducing new products. In Europe, especially in the EU Member States, innovation can play a significant role in a dynamic growth of knowledge-based economy which is capable of creating new jobs, supports an increase in labour productivity and ensures social cohesion. Generally innovations in enterprises can rely on improvements of product, marketing or organizational. Over the past 20 years, the Polish food and beverage sector has undergone significant transformation. It is one of the sectors of the economy, which soon was reborn after the crisis related to the political transformation, becoming the important stimulator of economic growth. Through technological developments, technological, and organizational sector Poland came to the forefront of modern and innovative European food producers. This study shows how is changed level of innovation in Polish sector of food and beverage production in years 1998-2013. Source data for the research were secondary. Aim of the study was to determine the dynamics of changes in innovation in the food and beverage sector and comparing the level of innovation towards other industries in Poland. Obtained results showed increasing amount of innovation in the Polish food and beverage sector, but compare to other industries remained at a lower level.
Keywords: *innovation, food, beverage, innovation process*.

Introduction

The major sources of competitiveness of Polish food sector should include price advantage, which has been preserved in spite of price convergence process. Low price is the advantage of Polish food producers, but they will shrink, so it is necessary to implement such projects, which promote the formation of a sustainable advantage over its competitors. One of the factors thanks to which Polish food business operators may gain competitiveness is innovation. Innovation is an important element that allows the company to adapt to changing environmental conditions. Innovations decide on the pace and directions of development of the company. Innovations in the food sector are needed, of course, financial resources which provides the European Union. Polish food manufacturers use different programs to support innovation and reform in the company. Innovation in the food sector are needed, of course, financial resources which provides the European Union. Polish food manufacturers use different programs to support innovation and reform in the company. This program is called the Innovative Economy. The Programme Innovative Economy is one of six national programmes under National Strategic Reference Framework, which are co-financed from EU resources. This programme is directed mostly to all entrepreneurs who want to implement innovative projects connected with research and development, modern technologies, investments of high importance for the economy or implementation and use of information and communication technologies. The Programme aims at supporting innovativeness in its broad sense. This aid comprises both direct support for entrepreneurs, business support institutions and scientific entities providing entrepreneurs with high quality services, and

systemic support, ensuring development of institutional environment of innovative enterprises.

Materials and methods

The primary objective of this article is to present the issues related to innovation activities of food industry companies, as well as presenting some of its effects. In the paper I presented the percentage of food industry companies, which examined years have introduced innovations. The effects of innovative activities presented using data from the Central Statistical Office of Poland. Data on innovation in the food sector which took into account from the years 1998-2013. Cyclical surveys on innovation activities of enterprises, conducted in accordance with a standard, international methodology presented in Oslo Manual prepared under the aegis OECD and Eurostat, are, inter alia, the key source of information included in a scoreboard with results of innovation surveys in which the European Commission collected 25 indicators for monitoring the Innovation Union. The thematic section presented in the publication concerns innovation activities of innovation active enterprises as well as process and product innovative enterprises. The implementation of organisational and marketing innovations are also included in many breakdowns.

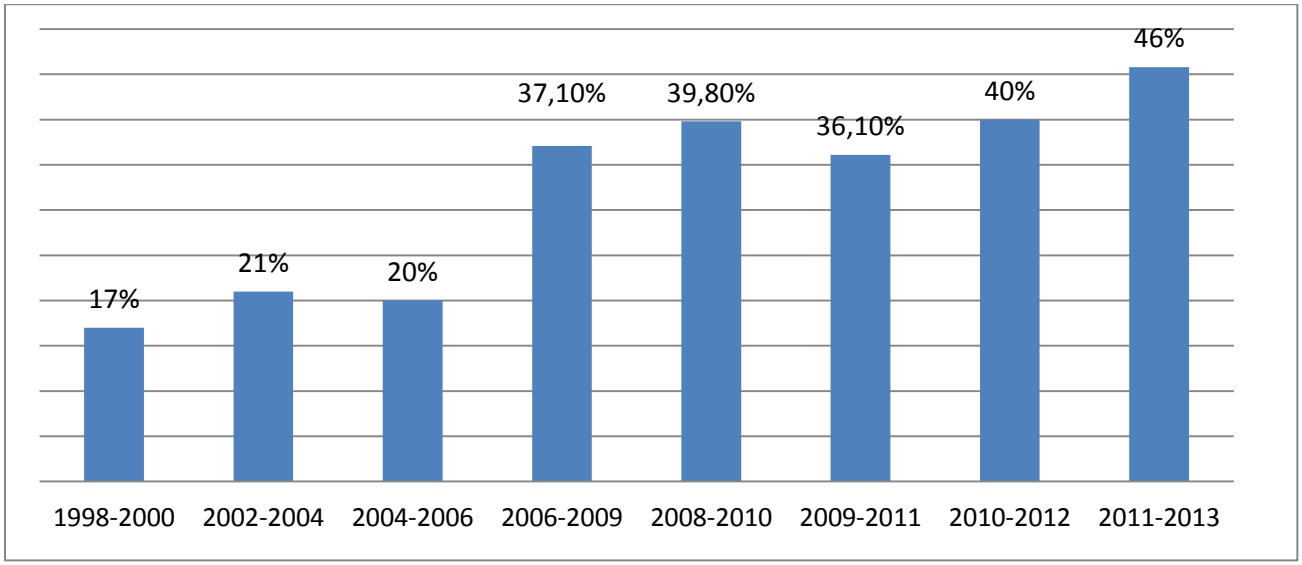

Figure 1. Industrial innovation active enterprises in the years 1998 - 2013 by NACE divisions - Manufacture of food products and beverages*
*source of information: Central Statistical Office of Poland

In the years 1998-2013 showed an increase in innovative activity of enterprises especially after the Polish accession to the European Union. Poland received massive financial support for the introduction of innovation in enterprises. Innovative activities embrace the activities of a scientific, technical, organizational, financial and commercial. As a result, Polish exports have increased in recent years. In 2009-2011 we observed a slight decrease in innovative activity of enterprises was caused by the economic crisis which also touch and Poland.

Results and discussion

In 2012, the value of production sold of the sector (excluding tax on goods and services and excise duties) amounted to 204.6 billion zł. (51,15 billion €). This corresponded to 21.4% of the sold manufacturing (which is one of the higher results in the European Union), and 18% of total industry sales. The sector employed 386 thousand people, equivalent to 18.9% of employees in the manufacturing sector and 15.6% of those employed in the industry. As you can see, then, the food sector is crucial for the Polish economy.

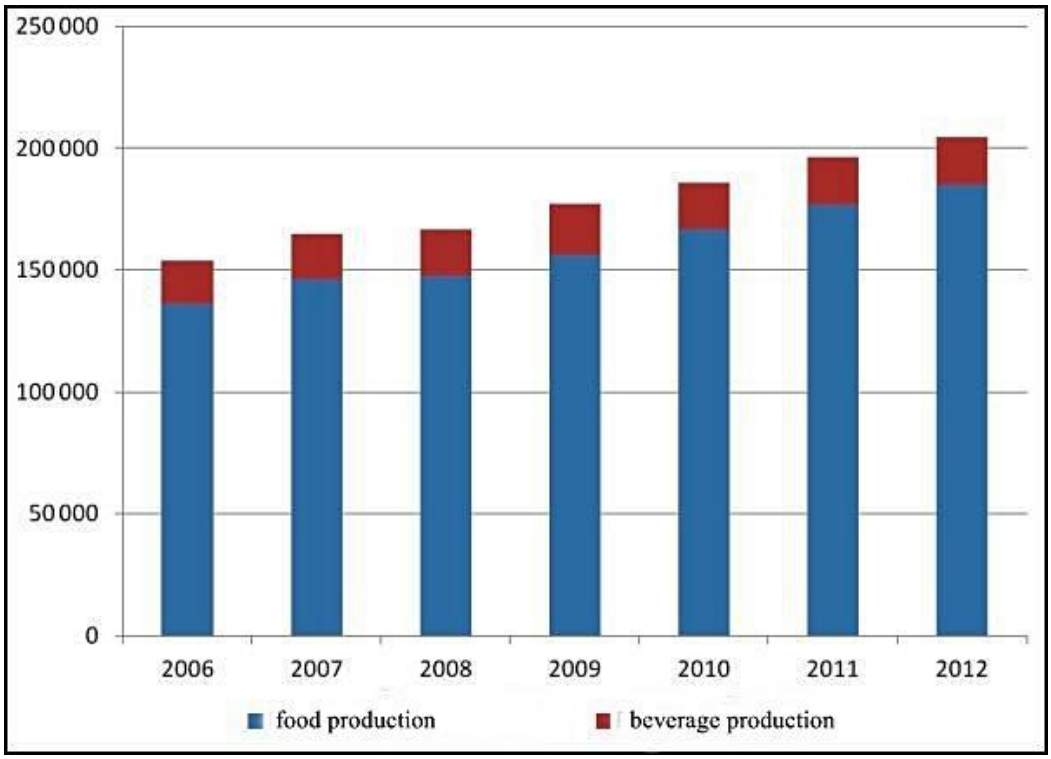

Figure 2. Value of production sold Polish food sector in the years 2006-2012. Own calculations *source of information: Central Statistical Office of Poland

Over the past 20 years, the Polish food sector has undergone significant transformation. It is one of the sectors of the economy, which soon was reborn after the crisis related to the political transformation, becoming the important stimulator of economic growth. Through technological developments, technological, and organizational sector Poland came to the forefront of modern and innovative European food producers. The result is a successful export of Polish enterprises. A major factor accelerating development of the sector was the Polish accession to the European Union in 2004. The reason was the large number of grants that allowed adaptation of plants to the standards required in the EU and innovation. The opening of European markets led to an increase in exports of agri-food products. Economic integration also meant the implementation in Poland of numerous foreign investments, which are an important source of innovation for the food industry.

Since 2004, Polish food exports increased more than fivefold, and trade surplus - thirteen. Despite the Russian embargo value of Polish food exports in 2014 to 2013 years increased by 4.5 percent. to 21.3 billion euros Of great importance they are also the Asian markets, as well as the Arab countries and North Africa.

Conclusion

Today, Poland is the eighth largest food exporter among the countries of the European Union. Our export hit are apples and mushrooms. We are the largest manufacturer in the European Union. These results would not be possible to achieve without strenuous effort to Polish farmers and exporters. These results would not be possible to achieve without strenuous effort to Polish farmers and exporters as well as innovation thanks to which Polish food has become competitive on the European market. Implementation of innovation has reduced manufacturing costs while introducing new products and technologies made Polish food is tasty, safe and competitively priced. Former President of the European Commission Romano

Prodi has stated that Bosnia and Herzegovina has a chance of joining the EU soon after Croatia, but it is entirely dependent on the country's progress. In the future, perhaps Bosnia and Herzegovina will be able to use funds from the European Union in this country because it is the potential similarly to Poland

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COFFEE COOPERATIVES PROMOTING PEACEBUILDING AND SOCIO-ECONOMIC DEVELOPMENT OF FARMERS IN HUYE DISTRICT, SOUTHERN RWANDA

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Abstract

Cooperative organisations play an important role in the peacebuilding efforts undertaken in the wake of the Rwanda genocide in1994. The genocide tore apart and destroyed large parts of the country's socio-economic and institutional foundations, its consequences are still evident. Coffee is the main source of income for approximatively 500.000 households. The paper aimed to analyze the impact of coffee cooperatives and the coffee washing stations in the peacebuilding socio-economic changes of coffee farmer's members in Huye District, Southern Province of Rwanda. A research surveyed selected 70 small coffee farmers from 3 cooperatives to measure socio-economic development of coffee washing stations on the growers. Results reveal that cooperatives increase production and improve the socio-economic households' income (86.5%). Jobs are created for women and girls especially during the harvesting and the coffee processing (98.7%). When farmers are working together, a synergy is created among them, by disucussion, exchanging experiences, which avoide discrimination, conflict, division between antagonist ethnic groups Hutu and Tutsi (67.1%). Results reveal that coffee cooperatives promote a spirit of understanding each other and the tolerance between farmers (72.3%). Cooperatives provide loans to the members through the "rotating funds: *Ibimina*". They also educate members through vocational trainings by enhancing and empowering farmers especially women and girls (97.1%). This is evidenced by the provision of adequate shelter, the observation of human rights, access to the medical insurance, school fees for children, undertaking entrepreneurial activities, unity and reconciliation between members, participating in the household's incomes allocation. Cooperatives uses various strategies in peacebuilding such as working together to construct new schools and houses, land inheritance for women, clubs for peacebuilding and visiting each others (74.3%).

Keywords: *coffee co-operative, coffee washing station, peace-building, socio-economic development, Rwanda.*

Introduction

The 1994 genocide in Rwanda tore apart and destroyed large parts of the country's socio-economic and institutional foundations, and its severe consequences are still evident. Cooperative organisations serve as a means to strengthen social cohesion and facilitate reconciliation and recovery the values of Rwandans especially while farmers are cultivating together or when they are treating their coffee cherries (Gisaro, 2013). Rwanda's economy is largely agriculture based. This sector provides above 61% of export revenues, principaly from coffee and tea (NBR, 2014). There are about 800 millions coffee plantations belonging to 500,000 households producers in Rwanda (OCIR-Café, 2013, Ngabitsinze, 2012). As the years go by, coffee production steadily decreased both in quantity and quality. National strategies focused on liberalisation of the coffee value-chain started in 1998, strongly invested in creation of Coffee Washing Stations (CWS) accross coffee productive areas and grouping

coffee growers into cooperatives. The purpose was to produce a fully washed coffee, high quality coffee for international market which is able to offer better income. The CWS were put in place and are justified by socio-economic advantages and services offered by coffee cooperatives. These advantages include the increase of coffee prices and revenues, access to proximity extension services, creation of jobs, access to health services, access to education,credit and better resettlement, markets amongst others. Based on the aforementioned statements, the following research questions were formulated: (i) Do the CWS and the cooperatives activities have significant impacts on the welfare of coffee growers? (ii) To what extent coffee cooperatives through their CWS improved peacebuilding and the socio-economic development of coffee producers?

Materials and Methods

Data were obtained from a field survey in Huye coffee cooperatives both based in Huye District in the mountain hills of Huye, Southern Province of Rwanda, from March to June 2014 during the coffee processing. The cooperatives AKM, HMC and KOAKAKA where taken into our study. All primary and secondary sources of data were used. Primary data were collected through interview, questionnaire and focus-group discussions. Interviews were conducted with management committee of the selected multi-purpose cooperatives and CWS. The questionnaire was administered with randomly selected 70 members of the selected coffee cooperative organizations. Guided by a purposive selection of respondents and based on a certain number of criteria previously set: (i) to be a coffee grower, having a minimum of 150 coffee trees with an experience of more than 5 years in coffee farming system; (ii) to prove a seniority of at least 5 years in the cooperative (iii) to have 3 dependent persons at the time of the investigations; (iv) to show evidence of an active involvement with the cooperative and to deliver the cherries to a CWS; (v) to be member of one of peace clubs at the cooperative level and to be an active participant in it is activities. The three cooperatives counted for a total of 2,811 members, of which 1,353 were from AKM, 142 from HMC and 1,316 from KOAKAKA. Interviews were done from 12 key informants especially the managers of the CWS and the cooperatives. The collected data was analyzed using descriptive statistics such as mean and percentages with regard to the different socio-economic variables provided by CWS and cooperatives.

Table 1. Sample with the categorization of the households by zone and by cooperative

AKM			**HMC**		**KOAKAKA**			
Category of growers		**Freq.**	**Growers**	**Freq.**	**Growers**	**Freq.**	**Total frequency**	**%**
Cat A	50% x 32	16	50% x14	7	50% x 24	12	35	50
Cat B	40% x32	13	40% x14	6	40% x 24	10	29	41
Cat C	10% x32	3	10% x14	1	10% x 24	2	6	9
Total		**32**		**14**		**24**	**70**	**100**

Source: Data from the survey, June, 2014.

Results and discussion

Economic benefits of the CWS and the cooperatives

The liberalization of the coffee sector has had a number of positive effects. The socio-economic impacts of the CWS and the cooperatives on the producers follow from the advantages and services offered to the coffee growers. Farmers have an incentive, increased income, to shift some production from semi-washed to fully washed coffee. Results reveal that a number of direct financial benefits, such as increased prices received for their cherries, employment opportunities, and better and easier access to loans, particularly access to credit

to purchase inputs such as fertilizer and agricultural inputs. Farmers also noted that their families are now better fed, that they are able to hire laborers, that they have help with marketing and sales, and that they receive some medicines for free. Farmers also reported that they benefit from access to coffee bicycles to transport coffee cherries and other productions. Less directly, farmers stated that they benefit from socializing with and learning from others. They felt their work was now easier (they no longer process cherries at home) and that they could spend time on other home activities. The prices given to the producer evolved irregularly with an average of 362.2Rwandan francs (Rwf) per kilo (0.4US$) against 460 to 500Rwf/kg that the CWS offered the speculative trader. (Figure1).

Figure 3. Prices of green coffee on local and international markets (2002-2012).

The treatment of coffee has valorized its quality through sellings (from 450Rwf/kg in2002 to 1.100Rwf/kg in 2010). With the presence of the CWS the producers received almost 3 times the prices of coffee on the local market. On the international market, the prices went up progressively. From 2002 until 2010, the mean prices was 2.71 times higher than local ones, with two extremes to the rise and to the decrease in 2006 (3.84 times passing from 527 to 2.022Rwf) and in 2005 (902 against 1.910Rwf, 2.11 times the local prices).

The Coffee Washing Station: source of jobs in the rural areas

The functionning of the CWS come with the creation of jobs by employment of a permanent staff and technical support, mainly during the harvesting season. The manpower teams (daily, occasional and seasonal) are reinforced every campaign in the activities of picking, transportation, sorting, treatment, drying, storage and marketing of the green coffee. The created jobs contribute to sustain the income and the perspectives of future especially for the vulnerable groups. These are notably women, widows and most abandoned social populations (98.7%). They are often victims of the discrimination and do not have access to the non-agricultural jobs. The treatment of cherries valorized the women's activities (89.4%). Through their activities the CWS showed evidence of gender integration in their daily tasks during the coffee campaign. Thus, they are the good way to promote gender and to give voices to the women and girls in rural areas of Huye District.

The dynamism of micro-finance activities through CWS and cooperatives

In Rwanda, farming world and the coffee production were not judged attractive for the financial institutions, due to the lack of loan guarantee. The cooperatives accelerated the

emergence the micro-finance programs into funds mobilization. Thus, CAPEC[26] micro-finance opened in Maraba sustains the activities of small income generating projects for members. The monthly interest rate is from 1 to 1.5% according to the kind of activity. The guaranty is solidarity based on the relationships and the mutual confidence between the members, thus constituting a pledge of guarantee to obtain the credit. The possession of a bank account number became obligatory to all members for the payment. Having a bank account has an economic and social advantage: the culture of saving, a sign of social elevation, providing a better vision, a change of mindset. Savings enhance the self-confidence, a sign of group encouragement in the time of taking loan. These activities are increasing their daily income.

Coffee Washing Station: factor of technological transfer in the farming world

The setting up of the CWS by the cooperatives introduced new technologies. Additional facilities have been created in the domains of mini-hydroelectric power stations and the tele-center that have been placed exclusively at disposal by cooperatives in favour of their members. The tele-center of AKM (Kizi) helps the community to reach the new fashions of modern communication, to facilitate access to the outside world like searching of new outlets for their coffee. It participates in the trainings and offer internet-café, the tele-show as well as a canteen for the inhabitants of the zone. A lot of activities are exercised there, notably the movies projected on the maintenance and the follow-up of coffee, the domestic agriculture, the market gardening, the domestic scheduling, hygiene, the community health, the first-aid, the basic literacy. The protection of the environment, the balanced food, the struggle against the sexually transferable illnesses such HIV-AIDS, the struggle against the ethnic discrimination, the prevention of conflicts, the unity and reconciliation, children rights are also developed in the tele-center.

The contribution of the CWS to the balance of the payments

In 2000 there were only two washing stations in the country (Nkora and Masaka,) neither of which worked, actually there are now 230. This has resulted in 32% increase in production of fully washed coffee. Coffee prices for fully washed increased from 1.6 US$/kg in 2003 to 3.7 US$/kg in 2013 while in the same period ordinary green coffee prices increased from 1.24 US$/kg to 2.6 US$/kg. The activities of the CWS and cooperatives in relation with the export of fully washed coffee brought significant foreign currencies in the country. Thus, the penetration of the foreign markets is strongly remunerative. The contribution to the commercial balance intensifies while reducing the strong dependency vis-a-vis the only one semi-washed product. These changes have important effects on the ground in Rwanda. Coffee continues to generate important export revenue for the country. While figures vary year-to-year, the country’s coffee exports stand at 19,573 metric tons in 2014 generating over US$57 million in revenue, a leap from US$38 million provided in 1995. The generated revenue increased the national economy and has contributed in the development of the country and the coffee farmers especially (NAEB, 2013, Ngabitsinze, 2012, Boudreaux, 2011).

Coffee Washing Station and its effects on the environment

The construction and the functionning of a CWS either improve or destroy the environmental conditions of its perimeter. The waste water released after fermentation in the environment

[26] Caisse Populaire d’Epargne et de Crédit.

constitute source of pollution. The solid organic matters constituting the pulp of coffee are decomposed specially designed pits. After decomposition, the pulp can be used as a fertilizer, rich in mineral elements, as it is the case in Maraba. In Karaba, on the other hand, the waste waters and the pulps of the Karambi station are not treated, recycled and poured in the pits that are destined to them. Therefore, they pollute the environment of the riparian populations and the food crops localized on the watershed of Kibingo river near Karambi for example. The management of a CWS should plan specific systems to control these sources of pollution efficiently, while treating the pulps of coffee useful for fertilizing farms. The results are significant in terms of essential compost manure for agriculture (65.3%).

Direct effects of the Coffee Washing Station

The CWS helped to produce a high quality coffee marketed at highly competitive prices. It improved the livelihood of the producers through monetarization of the farming economy as well as the technology transfer. They facilitate the extension actions in favour of coffee innovations through trainings to the members. Other advantages include (i) Giving chemical fertilizer (24.9 %), 22.8% for pesticides, insecticides, herbicides 22.4% provide seeds; (ii) The reduction in labour demands required in coffee processing activities for the vulnerable groups: households headed by children, widows and aged people (98.2%); (iii) The creation of jobs especially for women; (iv) The facilitation of the access to micro-credits (21.9%); (v) The access to electiricity and agri-inputs, the income generated by the sales of coffee which accumulate the capital and translated into signs of wealth within the households (76.5%).

Socio-economic advantages of the coffee cooperatives

Economic advantages

The coffee growers adhere with the purpose to protect their interests, to find satisfaction of their needs: better conditions, pecuniary advantages and various other services. Others adhere to get the facilities and agricultural inputs offered through the cooperative. Consequently, the members desire to acquire strength and the necessary synergy to improve their well-being.

Increase of income and purchasing power

The speciality coffee industry in Rwanda, has helped to change lives of the people. By growing better quality coffee, producers are earnings more. Higher incomes benefit farmers, their families, and their communities in a variety of ways: farmers can improve a home, pay medical expenses or school fees, or better ensure food security. When cooperatives earn a profit this allows them to hire workers, purchase capital, and support community projects such as improved schools. The cooperative helps to increase the incomes of its members. The producers are motivated and invest more in the production of high quality. The remunerative prices serve to improve their living conditions. The cooperative allows to the members to access inputs and agricultural facilities and to develop business spirit.

Negotiation power

The CWS through the cooperatives acquired a real power of negotiation with the local authorities, the charity organisations, and the rural development projects nearby. Some financial institutions are henceforth present in the farming zones of Maraba and Karaba since

2003, notably the Popular Bank of Rwanda, the micro-finance institutions, and the SACCO[27]. Belonging to a cooperative and participating actively in its activities, constitute a pledge of reliability and durability vis-a-vis the direct beneficiaries and it is supported by internal and external partners. The CWS and the cooperatives are therefore considered like important vectors and indispensable engines for the development of the farming world in the zone under survey. These organizations are important business entities in enhancing the economic condition of their members by improving their bargaining power and income.

Peacebuilding among social advantages of the cooperative

The cooperative create strong social ties which constitute a very important social capital for the members. These ties concern style of the social cohesion, mutual help, interrelations, courage, confidence, trainings, entrepreneurship, auto-creation, and enterprise mindset, amongst other. The adherence to the cooperative permitted a full bloom of the members while giving them the opportunity to exchange between them, to put forward themselves in the society and to assume their responsibilities. Often, these actors undertake in common social type activities (e.g. IBIMINA[28]) and economic activities outside the framework of their cooperatives. The cooperative helps also to tighten the ties of solidarity, involving often other family's members (58.4%). The cooperative reinforces the unity and reconciliation of the members torn and divided by 1994 genocide without distinction of sex, ethnic group, origin, religion, etc. (66.7%). The cooperative taught them to forgive mutually in order to reinforce the unit and the reconciliation (61.5%). Working together, farmers have new opportunities to interact with other. These repeated interactions may be helping to lessen the sense of ethnic distance among members of Rwandan’s society (56.1%).

Access to credit through cooperative

Source of credits and their use by the members

The loan constitutes an advantage for the members to join the cooperative. 87.8% of the respondents want to acquire credit against 12.2% that abstain themselves due to lack of repayment means. Most interviewees (91.6%) prefer to solicit the credit cash against 6.1% who wish to have phytosanitary products, and 2.3% agricultural facilities. The credit serve to cover the urgent needs: soldering, the purchase of seeds, the school supplies, emergencies such as illness. The guarantee is bound and is related to the harvest and the personality of the credit seeker. Cooperative helps member to benefit of fertilizers through the National Agricultural Export Development Board by refund during the harvesting period.

Table 2. Prices of fertilisers in Kizi/Maraba Market, June 2014(in Rwf/Kg).

Types of fertilizer	Coffee growers	Quantity (Kg)	(%) of coffee growers	Mean price/Kg	Total cost (Rwf)
Urea	5	60	6.25	490	29,400
DAP	3	36	3.75	600	21,600
NPK	72	1,119	90.00	560	62,640
Organic matter	80	984,727	100	-	-
Total	**80**		**100**		**677,640**

Source: Our calculations from field survey, June 2014.

[27] Saving and Credit Co-operative.
[28] IBIMINA : Tontines established by peasants, functioning as farmer’s association groups.

Factors hindering the coffee growers access to credits

The factors that limit the access to the credit are generally the uncertainty of the production (31.2%), the bad design of the project (20.0%), the lack of guarantee (17.5%), the high administrative cost (15.0%), the complicated procedures (8.8%), and the ignorance on the procedures to access the credits (7.5%). The cooperative prepared their members on how to access to the credit, contrary to non-members that solicit credits from the nearby banks.To overcome the blockages of access to the credit, the cooperative acts as an intermediary between the grower and the bank who bestows the credit. It plays the hinge role in favour of the members. The repayments are made by the cooperative, the organization of relay that subtracts the amount of the credit when the coffee cherries are delivered (Fig. 2).

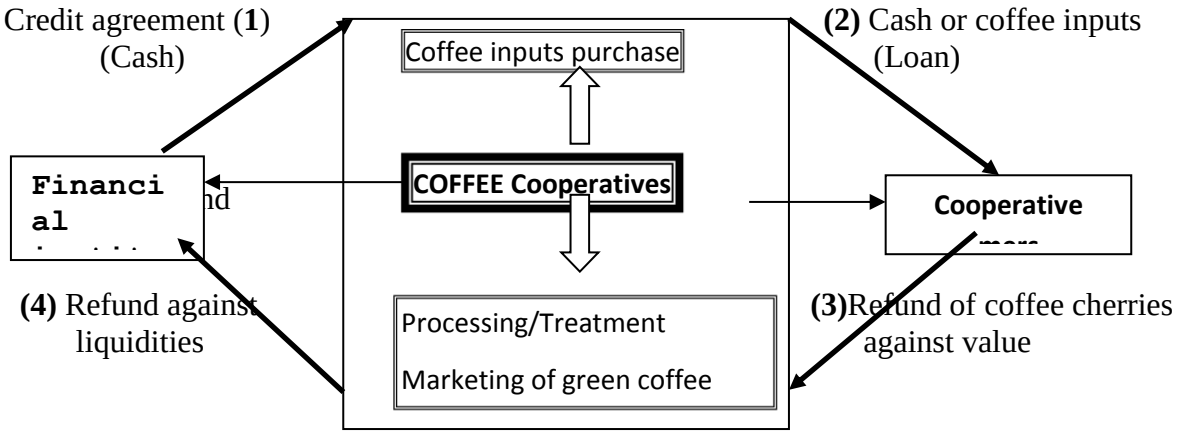

Figure 2. Channels of the demand and repayment of the credit through the cooperative.

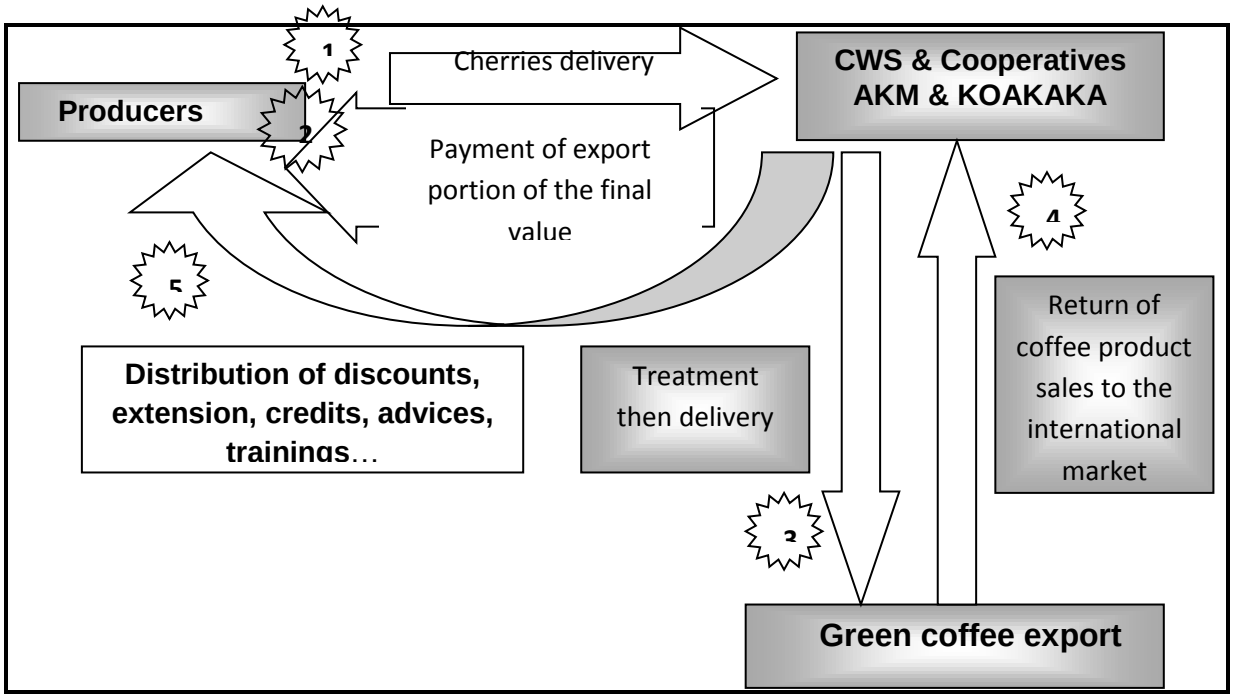

Figure 3. Process of sale and repayment of coffee through the cooperative.

The producer remains the main link in the production of coffee in Rwanda. Organized into cooperative, it is a means to benefit from the credit and other technical supports from NAEB. The producer delivering his cherries, receives part of its sale from the cooperative.

Conclusion

The coffee cooperatives and it is coffee washing stations has opened the perspectives of new jobs for the most vulnerable groups benefitting from no aid while especially fighting against poverty and the rural urban exodus of the young people. The jobs have provided income which has helped growers to improve their welfare which has the correlation to promote peacebuilding among members's families. Coffee became a visa to access cash credits, inputs or agricultural facilities. Through the credit-coffee system the coffee growers succeeded to pay eduction for their children. They benefit from a socio-economic advantages and services. However, the low level of education of the producers is a challenge to the growth of the cooperatives. The CWS and the cooperatives count a lot on outside supports (donnors and the state) instead of taking themselves in charge. Thus, they stay fragile without autonomy and because of their dependence. They are strongly competited by the private operators who buy, treat and export also the fully washed coffee. To guarantee the sustainability of their actions and the development of their members, the CWS and the cooperatives should position themselves better in order to be a lot more competitive on the market.

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OBSTACLES OF AGRICULTURAL EXTENSION WORK IN THE KINGDOM OF SAUDI ARABIA

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Abstract

The main objective of this study was to investigate the obstacles facing the Extension Workers (EWs) during their field work in the Kingdom of Saudi Arabia (KSA). The EWs' suggestions on how to face each obstacle. The study covered all the 67 EWs working in four Regions, namely: Al-Qassim, Najran, Asir and Jizan. Frequencies, percentages and averages were used for data presentation and analysis.

The most important results:

- The most important organizational and administrative obstacles facing the EWs, according to the average means were: insufficient financial incentives for extension worker; lack of allowances for the nature of extension work, insufficient physical facilities; small number of specialists in agricultural extension; incentives are not proportionate with performance.
- The most important technical and, production obstacles facing the EWs, according to the average means were: unavailability of farmers in their farms during the working hours, non-Saudi laborers are responsible of farms, relatively large number of farms are under the responsibility of EWs, inconvenient conditions of extension work, and overfocusing on the agricultural, rather than educational services.
- The most important EWs' suggestions to overcome the obstacles facing extension work were as follows: providing and supporting allowances and incentives, Increasing the number of EWs, providing both material resources for the extension work, appointing newly graduated EWs, redistributing and identifying work responsibilities, clarifying the functions of each department and each staff member, providing allowances and incentives, supporting specialists for extension work, liberating EWs from administrative work.

Keywords: *Obstacles, Agricultural Extension Worker, Saudi Arabia*

Introduction

Agricultural extension agency in the Kingdom received particular attention by those in charge of the Ministry of Agriculture, including the provision of support and budgets required for work, in addition to support of the agency with the qualified extension staff of specialists and extension workers and providing the suitable training to them through the specialized training courses or delegation for higher scientific degrees in the field of agricultural extension. The agricultural guide is a key element of the extension agency because he undertakes several roles and duties. Therefore, all types of material and human support of the extension agency are valueless unless the extension workers are highly efficient and effective (Okley and Garforth, 1990). In consideration of the important role of the agriculture extension agency in the provinces and governorates of the Kingdom, and through the extension workers working close to farmers in the departments of agriculture affairs and agriculture directorates and branches that are about 13 agriculture affairs departments and 12 agriculture directorates, and about one hundred twenty agriculture branches, and the low number of academic researches on the key obstacles of agricultural extension, in addition to the fact that one of the

researchers is considered a key responsible for the agricultural extension on the central level of the ministry, so the problem of this research is summarized in definition of the key obstacles of agricultural extension and suggestions for addressing them in the Kingdom of Saudi Arabia.

The Arab Agricultural Development Organization (AADO) (2009) indicated in a study recently conducted by it in that a key obstacle that reduces the efficiency and effectiveness of work is the weak and lack of qualification of extension workers. El-Shafie and Emad (2009) indicated low number of extension workers who work close to farmers in Egypt. The study of Shaker and Al-Zaydi (2002) indicated that they key problems and difficulties that encounter the extension workers on setting the agricultural extension programs include the assignment of extension workers to works that are not in line with the nature of their work, and the weak coordination between the agricultural extension and agricultural development organizations, unclear job duties of extension workers, low training opportunities, work pressure on the extension worker over his capacities, limited opportunities of job promotion, some extension workers don't understand the planning measures and insufficient capacities of extension work. To overcome these problems, the researchers recommended to increase the opportunity of attending training programs, particularly in the field of planning of extension programs, and the extension workers' dedication to perform their extension roles and duties, supply of capabilities for implementation of the extension work plans, provision of the audio and visual extension aides, provision of material incentives of extension personnel, drawing attention to farmers' participation in planning of the extension programs.

The agricultural extension department in the Ministry of Agriculture, Kingdom of Saudi Arabia (MADAE) (2010) noted that the obstacles that encounter extension work in the Kingdom include the low number of technicians working in the general departments and directorates of agriculture, failure of extension worker to be dedicated to the extension work, lack of farmers' attention to the symposiums and meetings organized by the agricultural extension agency, need to qualification and training of extension workers and reliance on the theoretical and applied approach combined, farmers' lack of understanding of the role and importance of extension worker, agriculture farmers' lack of understanding of the Ministry's direction in respect of the farm affairs due to the farmers' lack of presence in their farms.

The primary objective of this research is to examine the key obstacles of agricultural extension works and the means of addressing them in the Kingdom of Saudi Arabia through the following secondary objectives:

Determination of the key administrative and technical obstacles of agricultural extension work in area of the research.

Exploring the personal, economic and social traits of the respondents, including: age, place of residency and establishment, job title, education, job grade, salary, major, years of experience in agricultural work and extension, period of study of the extension courses by the respondents and its sufficiency, the respondents' enrollment in training programs, job satisfaction, extension workers motivation for completion in the area of the research.

Identification of the suggestions of extension workers on who to overcome the obstacles that encounter them.

Material and Methods

This study was conducted in the areas of Qassim, Assir, Jazan and Najran in the Kingdom of Saudi Arabia, due to their agricultural importance and position in the Kingdom. These areas meet the suitable agricultural capacities that make them the best areas of the Kingdom in terms of agriculture such as water, environment and lands suitable for agriculture. In addition, these areas found attention and care of the state. Several establishments and agricultural branches of government were established. Four agricultural affairs departments were

established with forty branched. The agricultural loans by the Agricultural Development Fund for farmers in the four areas in 2007/2008 were approximately SAR 277 million that represent about 27% of the loans provided in all regions of Saudi Arabia (Saudi Arabian Agricultural Bank, 2007/2008: p. 25). The research community includes extension workers who work in Qassim, Assir, Najran and Jazan (n= 67).

Procedural definition of the study:

Extension worker: every employee who works in the section of agricultural extension in the general departments and directorates or agricultural branches, and he assumes the extension duties whether the job title is specialist, researcher, engineer, agricultural technician or any other nomination.

Job satisfaction: The overall attitude of extension workers with their work. It is measured through 23 sentences.

Motivation for achievement: This is set of factors that motivate the individual to be good at his work, and it is measured through 23 sentences.

Obstacles: these are the difficulties and problems that encounter the extension work from the extension workers' perspective.

Data Collection:

The researcher depended on the post questionnaire as a means for data collection, and respondents were followed up through the telephone calls to complete or confirm some data. The questionnaire included many questions that serve the objectives of the research. The questionnaire form contains four sections. Section one is related to the personal, economic, social and job properties of the extension workers, such as: age, place of birth, place of residence, education, specialization, job title, grade, years of experience in agricultural work, years of experience in extension work, pre-service training andpost-service training. Section two is related to the scales of job satisfaction and motivation for achievement. Section three handles the obstacles that encounter the extension workers during the performance of their extension duties and how to overcome them from their perspective.

The repetitions, percentages and arithmetic means were used to present and analyze the data.

Results and dicsusion

Impediments of agricultural extension work and proposed solutions: Data of the study reveal many problems and difficulties, including technical, administrative, production and organizational problems and difficulties that encounter the respondent extension workers during their undertaking of the extension roles and duties. The results show that there are problems and difficulties related to factors out of the extension regulation that may be related to social and environmental sides.

the key organizational and administrative obstacles that encounter extension workers in the area of the research according to the arithmetic means are: low material incentives of the extension worker, lack of any cost of living allowance, low material capabilities in the extension system, lack of extension specialists in the extension system. The incentives provided to extension workers are not related to the performance level, by arithmetic means of 2.75, 2.73, 2.61, 2.51 and 2.47 respectively. These results assure the importance and effect of the factors related to the material sides for the respondent extension workers. The respondents considered these sides are the key obstacles that reduce the efficiency and effectiveness of agricultural extension work. These results may be attributed to the fact that officials in the Ministry of agriculture don't understand the rule and importance of the extension worker's work. Unlike their colleagues in the other government sectors that provide many incentives and allowances that may constitute 25-50% of salary, no incentives or allowances are allocated to them. In addition, it can be interpreted based on that the ministry's system doesn't

differentiate the white collars and blue collars under the hard weather and topography conditions. In addition, the increase of income has become a key matter that any employee seeks under the high inflation and rise of the costs of living in the Kingdom of Saudi Arabia.
The least important organizational and administrative obstacles from the respondent extension workers are: "strategy of extension work in the ministry is indefinite", "responsibilities and duties are unclear and indefinite", "double supervision of extension workers", "multiplicity and conflict of instructions given to the extension worker", "unclear of concept and objectives of extension work of extension workers", by arithmetic means of 1.76, 1.75, 1.75, 1.71 and 1.62. The low importance of these obstacles, from the perspective of respondents, is due to their satisfaction with the directions of the Ministry of Agriculture to restructure and organize the agricultural extension system on all administrative levels in the Kingdom, in addition to their conviction with the possible control of these administrative and organizational obstacles.
The key technical and production obstacles that encounter the extension workers in the area of the research, according to the arithmetic means, include: "farmers are not present in their farms during the official work hours", "it is the expatriate workers who assume the affairs of agriculture", "large number of farms under responsibility of the extension worker", "work conditions are not suitable to the job requirements" and "focus on the agricultural, not educational, services", by arithmetic means of 2.69, 2.67, 2.41, 2.33 and 2.33, respectively.
Moreover, the least important technical and production obstacles from the respondent extension workers' perspective are: "low attention to the evaluation and follow up of extension programs", "work out of the specialization", "there is no implementation plan to apply and achieve the strategy and purposes of agricultural extension", "unclear extension duties and tasks performed by the extension worker", "farmers' lack of appreciation to the extension worker", with arithmetic means of 2.10, 1.95, 1.89, 1.73 and 1.70, respectively. These results confirm the necessary attention to all obstacles without focusing on one obstacle. Importance of these obstacles may vary in accordance with the economic and psychological conditions of the extension workers who work in the agricultural extension system.

Suggested solutions for the obstacles that encounter the agricultural extension work:

The key solution suggested by the respondents to the obstacles that encounter the extension work include: support and supply of material incentives (mentioned by about 36% of them), followed by the increase of the number of extension workers (about 31%) and supply of material capacities, increase of the number of extension workers/ appointment of new extension workers (about 27% for each solution), coordination with research centers (25%), supply of the required work facilities, increase of the number of extension workers (about 23% for each), distribution and definition of the work and responsibilities, indication of the duties of every section and every employee (about 22% for each), supply of incentives and allowances, support of the extension staff with specialists,. Dedication of the extension worker to the extension work (about 20% for each).
The agricultural extension staff can be expected to reach satisfactory levels for those in charge of the Ministry of Agriculture whenever these solutions are considered in terms of the importance of implementing them in reality, and belief of the officials in the Ministry of agriculture with all its sectors that the extension services and agricultural extension work are the key works on which the Ministry shall focus, because it is the means for promotion of agriculture in the Kingdom of Saudi Arabia.

Based on the results of this study, some recommendations can be suggested to contribute alleviate or eradicate the obstacles that encounter the extension work in the Kingdom of Saudi Arabia as follows:

Holding training programs in the different fields of agricultural extension to bridge the knowledge and skill gap in the extension workers. It is preferable to hold these courses for the new entrants to the extension service and the extension workers who are actually in service.

The job description and definition of the duties and competences of workers in the agricultural extension agency in the way that guarantees the increase of efficiency and effectiveness of extension work.

Fixing and releasing suitable material allowances and incentives to extension workers who work in the field.

Creating the extension worker ‘s job within the jobs of those working in the agricultural extension agency because the title “extension worker” doesn’t exist in the Kingdom.

Observe the scientific and social properties of the extension worker such as the place of establishment, residency, qualification and specialization of agricultural extension on the appointment of the extension agency.

Increase the material and human capacities of the agricultural extension staff.

Increasing coordination and cooperation with the professors of agricultural extension in universities so that they assume the consulting role in relation to the extension work and its development.

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BUSINESS NETWORKING - CONDITION TO INCREASE INNOVATION IN AGROSECTOR OF SERBIA

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Abstract

The paper emphasizes the importance of business networking agricultural enterprises, whereby creating favorable conditions for their business and achieve a positive operating result. By combining can create new resources which are difficult to copy, and so pooled funds contributions and creating greater value of the final product. The paper points to some forms of business networks (business incubators, cooperatives, clusters), noting and their characteristics. In Serbia, the largest number of clusters are formed in the area of agriculture and food industry. The paper analyzes the definition of business networks, the types of business networks and how they manifest in the agricultural sector, or whether the population is willing to accept changes or continue to believe that the problem is unsolvable nature. Then, provided definitions of forms of business networks of the respective authors and have been isolated associations that are important for agrosector of Serbia. The aim is to determine the degree of crosslinking of agriculture in theory and practice, a willingness distrustful population and businesses to come together to bring about change in agriculture, so that in the future time will improve better image of national agriculture and complete economy. Research methods are based on search literature and web sites that highlight the importance of innovation and business networking in agriculture.

Keywords: *business networking, clusters, innovations, agrosector.*

Introduction

Association of producers and their joint appearance in the market encourages innovations in the agricultural sector, because only joint forces could create a favorable business environment for the production, processing and marketing of agricultural products, with constant innovation of the whole production and manufacturing process.

Stefanović et al. (2012) point out that it is important to make a distinction between the concept of invention (scientific or technical discovery, and always are linked with creation) and innovation (a new way of putting old and new ideas to realization). You should then approach the planning opportunities to some innovative ideas are realized by pooling agricultural producers, because the formation of business networks can lead to financial support of the Government of Serbia. More specifically, the Ministry of Commerce in December 2006 has done the program for the development of business incubators and clusters in the Republic of Serbia 2007-2010 in order to allow all producers in Serbia stimulate the association.

Studies in developed countries show that for small and medium enterprises a key factor in improving innovative capacity of involvement in different types of networks, especially clusters. The efficiency of activities and processes that are implemented in the network increases the chance that the company successfully used the opportunity to acquire new knowledge and its transfer through the network. Business incubators, cooperatives and clusters are some forms of business networking companies and agricultural producers. The common characteristic of these business networks is cooperation and connection of all member states. The largest number of formed clusters is in agriculture and the food industry.

Materials and methods

Research paper analyzes the possibilities of increasing innovation of the agricultural sector in Serbia, which, among other things, can realize business networking and association of farmers. This paper consists three parts. The first part discusses the definition of business networks by many important authors, as well as possible reasons association of farmers in the group. The second section discusses the types of business networks of agricultural sector in Serbia, where appropriate: business incubators, cooperatives and clusters. The third part gives concrete examples of business networks in the agricultural sector in Serbia from all the above forms of association. In the study data were taken from the works of local authors, from the report of United States Agency for International Development (USAID), but also with sites of individual associations and organizations.

Forming of business networking

Changes in the market position of individual participants lead to substantial changes in the operations of many companies. For companies it is important connection (association), not only with consumers but also with the other participants. Accordingly, emphasizes the significance of networking companies in order to increase their own innovation. Networking should not be only within the company but also with participants from the region. Such networking contributes to achieving the common goal of all participants - making a profit.

Studies in developed countries show that for small and medium enterprises a key factor in improving innovative capacity of involvement in different types of networks, especially cluster. Innovations in all areas efficient use and transfer of knowledge are key to the successful development of innovation networks. The success of the connection is conditioned by: the efficiency of the process in the network, structure of the network, reputation and ability of companies to take advantage of networking *(Stanković et al., 2011).*

The efficiency of activities and processes that are implemented in the network increases the chance that the company successfully used the opportunity to acquire new knowledge and its transfer through the network. In this way, create new resources that are difficult to copy. Continuous integration of resources of various actors involved in the network, on the one hand, increases their value and on the other hand, makes it difficult to use the resources of entities that are outside the network. The degree of their interdependence determines the density of the network. Benefits arising from the density of the network based on the exchange of valuable information and knowledge that is difficult to obtain outside the network. However, it is necessary to point to certain restrictions imposed by the network density *(Stanković et al., 2011)*:

sharing information and knowledge involved subjects and groups can sometimes reduce the ability of rational response the company and its strategic adjustment;

if some of the information and knowledge not been use in the right way, can affect the competitive advantage of enterprises.

Koprivica et al. (2009) defines an association as a group of individuals who voluntarily unite in the organization in order to achieve common objectives and programs in creating agrarian policy of certain areas sharing the risks and the benefits. The voice of farmers, regardless of the length and nature of the membership, are equally valid and all have the same rights. In most cases, members of the group are also, and neighbors, friends, relatives, people who have built a relationship of mutual trust and respect, and have collaborated on business without the need for legal and statutory regulations.

Stanković et al. (2011) state that in modern economic *reputation* of the network is a significant source of competitive advantage. Her positive image contributes to the successful positioning of all participants in the network. *The ability* of the parties involved depends on

whether the benefit arising from connection, to be used in the right way. *Adjustment of goals* is a very important activity in the development of relations and networks.
Last few years significant research efforts are focused on studying the role and importance of innovation networks. According to *Stanković et al. (2011)* some of the benefits of innovation networks are: *the mobility of resources, creating conditions for the establishment of new businesses, stimulate innovation, efficient development and transfer of technology and knowledge, developing new business models and access to markets, the globalization of business, better strategic and operational adjustment of goals.*

Forms of business networks of agrarian sector in Serbia

Enterprises of agrarian sector problems production and placement of its products faster and more efficiently be overcome with business networking. Implementation of programs to support the development of business networks is realized in 2007 (*Programme for the development of business incubators and clusters in the Republic of Serbia 2007-2010*).
The goal of business networks is to join forces which networked actors bring greater benefits than individual performance in the market. *Mihailović et al. (2009)* point out some forms of business networks: business incubators, cooperatives and clusters.
Business incubators enable the provision of direct support to companies that are just starting business, because in this period the most exposed to failure. Services that provide business incubators are offered at subsidized prices, which provides a convenient location to companies in the incubator as compared to those outside it *(USAID report).* Business incubators in agrosector of Serbia are not so represented and are still in the development stage.
Cooperatives of agricultural producers are based on the principles of modern cooperative movement. This concept can be applied to forming of a whole production chain. Cooperatives of agricultural producers, built on the principles of modern cooperatives movement, can be seen as a business system - company, with all of its business functions which would be implemented through a service or employment of appropriate specialities. For example, the organizational model of cooperatives of agricultural producers should be designed so that the fruit producers are not transported to the buying centers, but it submitted directly from the plot. The final destination may be refrigerator, some processing facilities or shopping centers *(Mihailović et al., 2009).*
Paraušić, Cvijanović (2014) agricultural clusters defined as a geographic concentration of small and medium sized enterprises, specialized suppliers, related services companies, research institutions and government agencies in the agriculture and rural development. *Džanković-Jerebičanin, A. (2014)* points out that the strategy of clusters in Serbia must begin with mapping, to identify local and regional competitive advantages. In Serbia, the clusters in the agricultural sector formed more in the form of associations, at the initiative of the enterprise, much more because of better positioning in the market, and less for obtaining state incentives.
However, *Radojević (2007)* states that there are so-called **quasi clusters (associations)** which have the perspective in the future to become clusters. For Serbia and the agricultural sector is important to associate in any form, which leads to non-compliance of laws, programs, systems illogical and high capital costs.

Examples of business networks in the agrarian sector of Serbia

In this section are discussed concrete examples of agricultural business incubators, cooperatives and clusters which function on the territory of Serbia.
As an example of the **agricultural incubators** in Serbia is Municipality of Novi Bečej, which all registered farms and young farmers under 35 years of making available land, greenhouses, buildings, equipment, training and mentoring. Greenhouses are provided in cooperation with USAID. Farmers themselves are realizing their production, and the incubator helps them to find a buyer, placement and charging product. After an incubation period, farmers will have to

continue to work on an agricultural holding by starting their own businesses and investments in raising of greenhouse *(http://www.dnevnik.rs/ekonomija/poljoprivredni-inkubator-sansa-za-biznis)*. According to the USAID report, business incubators that have started or are only registered in Serbia are: Incubator of Technical Faculties – Belgrade , Business incubator - Knjaževac, Incubator center – Niš, Business incubator center – Prokuplje, Business incubator center Bor – Bor, Incubator center or the development of entrepreneurship Rača – Rača, Business incubator Subotica – Subotica, Business incubator Zrenjanin – Zrenjanin, Business incubator – Senta, Business incubator Beočin – Beočin, Business incubator – Pančevo, Business incubator – Kruševac, Business incubator – Užice (http://www.skgo.org/bz/data/8%20LER%20Koncept%20Brosure/SER/Business%20incubators.pdf).
Given that small farmers can not enter into competition with large agricultural producers, for them is the solution to collaborate with other producers to make **cooperatives** to be very innovative and thus add value to their products. As forms of cooperatives in the agricultural sector occur in association of raspberry and cherry fruit cooperatives in Arilje, apple producers of Vojvodina. Thus, for example, the national association of fruit and vegetable producers *Plodovi Srbije* established in order to improve the production, processing, placement and joint appearance on the domestic and foreign market (http://www.naslovi.net/2008-03-05/emportal/prva-nacionalna-asocijacija-vocara-i-povrtara/593940).
Mišić (2007) considers that the term cluster is still unknown to the territory of the countries in transition. Our business people are more aware of other terms of association in agriculture: cooperative society, cooperative association, farmers' association or the chamber. Accordingly, the previously mentioned forms of association (baking industry, meat industry) are distinguished by the fact that the cluster approach, only those who know their business interests through a clear objective. Some of the goals of association in clusters may be *(Mišić, 2007):*
increase the competitiveness of domestic products in the domestic and international markets with an increase in exports;
efficient use of resources (natural, productive and human);
cooperation between businesses, educational and development institutions;
connecting with funds to finance innovative projects;
training and education, which are in the interest of the region and the government.
Bošković and Kostadinović (2011) point out that the largest number of formed clusters is in agriculture and the food industry. These authors distinguish the following clusters: Cluster *BIPOM* - Balkan-Black Sea agricultural machinery industry; *Šumadijski cvet* - cluster of flowers; POLUKS - Cluster of Serbia food industry. Clusters zero phase, who have organized themselves and work without the support of the Government is *cluster for revitalization of old crafts* in Serbia and Cluster *Somborski salaši*. *Džanković-Jerebičanin, A. (2014)* stated some clusters in the field of agricultural production and processing: Cluster *Agroindustrija* - combines a network of agricultural producers and processors of primary agricultural products Northern Backa; Cluster *AGRO START UP* - a network of companies for production and processing of agricultural products and health food; Cluster *PEKOS* - networks of enterprises for the production of bakery products and educational institutions responsible for supporting the work and development of clusters (raw materials, transport, marketing, education).
In order to improve current practices and find solutions to specific problems farmers can voluntarily and independently to decide for different forms of organization. Some associations are specialized for solving only one kind of problem, such as associations of fruit producers, beekeepers, vegetable producers or associate in mechanical engineering community that achieves significantly better technical equipment and the rational use of mechanization *(Koprivica et al., 2009).*

Conclusion

In modern business conditions, innovation is one of the main sources of competitive advantage. In the long run the only reliable basis for the development of the agricultural enterprises to innovate faster, better and more in relation to its competitors. This applies not only to the product/service innovation, but also increasingly on innovation processes.

Agricultural population of Serbia is old and difficult to accept change. They have difficulties in making the aging population convince that the introduction of innovations in their pursuit of agricultural activities give positive results. Even harder to convince them that their inclusion in the network will bring better business conditions, better living standards and a better picture of the future. Also, innovations in agriculture could partially retain or recover a young population which migrates to the cities in the demand for a better life.

The development of business incubators, cooperatives and clusters in agriculture of Serbia would create the possibility of increasing the competitiveness of not only this sector but also the whole Serbian economy. Note also provide financial support to farmers with undeveloped and marginalized regions of Serbia.

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FORMS OF RURAL TOURISM IN THE REPUBLIC OF SERBIA

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Abstract

According to the motives for which tourists come to the rural areas, forms of rural tourism can be defined. It is estimated that today there are over 50 different forms of rural tourism. According to the Organization for Economic Cooperation and Development (OECD) agritourism is the most common form of rural tourism in the European Union. The aim of this paper is to present the current forms of rural tourism in the Republic of Serbia and to highlight opportunities for their development. The main hypothesis is that agritourism is most widely spread varieties form of rural tourism in the Republic of Serbia. In order to prove the thesis, survey was realized on the 104 subjects of rural tourism in 11 districts in the Republic of Serbia (North Bačka, South Banat, Kolubara, Mačva, Moravički, Pirot, NorthBačka, Srem, Šumadija, West Bačka and Zlatibor). The sample was formed by random sampling. Due to the fact that in Serbia there is no register of rural tourism households, in order to form the sample, it is used a database of the National Association "Rural tourism of Serbia" as the most comprehensive. The research which was carried out included 10.95% of the total number of providers of rural tourism in the Republic of Serbia, according to the specified database. The instrument used in the study was questionnaire. The questionnaire was administered by interview. The main groups of questions were related to the characteristics of rural tourism operators and determining whether there is a link in the development of tourism and agriculture, as well as complementary services.The paper used qualitative and quantitative research methods inherent in the social sciences.

Key words: *rural tourism, development, the Republic of Serbia*

Introduction

Rural tourism includes all tourist activities that can be implemented in rural areas. Rural tourism has started to intensively develop at the European area in the 70's of the twentieth century, as a part of the strategy of development of rural areas. The development of rural tourism was the result of reduced tourism demand for mass tourism and more prominent demands of tourists for differentiated tourism offer. According to the motives for which tourists come to the rural areas there are defined forms of rural tourism. It is estimated that today there are over 50 different forms of rural tourism. According to the OECD agritourism is the most common form of rural tourism on the territory of the European Union. The forms of rural tourism, the length of stay may be divided into residential and rural tourism excursion. The common characteristic of all forms of rural tourism is seasonal demand, which causes low occupancy at the annual level. The average occupancy rate in rural tourism in the territory of the European Union is 25% (Bartlet, 2006) and in the Republic of Serbia is only 4%.

The basic tourists' motive in agritourism is learning about the life and work in agricultural households. In addition to the agritourism in the area of the European Union, the most common are the following forms of rural tourism - ethno-tourism, eco-tourism and wine tourism. Ethno-tourism and "tourism based on heritage," as it is called (Brooks, 2000),

represents the trip due to natural, historical and cultural attractions, or because of a desire to study the past, but also looking for an answer how local and regional art, the natural environment and culture influence on the present. According to the cited author it is a form of rural tourism, which is based on heritage and an integral part of the new economic strategy that aims to focus on the development and promotion of cultural, historical, geographical and natural resources, community or region. Ecological tourism or ecotourism represents a trip to the protected natural areas "for enjoying nature and other cultural values" (Novković et al., 2012). The development of eco-tourism could be linked to the agritourism, which would create tourism product that would be interesting to both domestic and foreign tourists (Radovic et al., 2011).

Wine tourism is an extremely popular form of rural tourism, which is usually associated with gastronomy and is together called eno-gastronomy tourism. Wine tourism includes tourist trip with a visit to the vineyards, wineries, wine festivals and exhibitions of wine, with wine tasting and/or experience attraction vineyard region represents the primary motive for the trip. Wine tourism in Europe is mostly developed through the wine roads. "Wine Trail is a special form of selling wine, hospitality, tourism and agricultural products of a winemaking region, and the tourist offer includes wine trail and closer tourist attractions that are not directly on the route, but they increase the ethno-tourist offer" (Pivac, 2012). It is necessary that the wine path contains "scenarios for guests, as well as organizing various events" (Kušen, 1999). The aim of this paper is to present the current forms of rural tourism in the Republic of Serbia and to highlight opportunities for their development.

Materials and methods

The aim of the paper is to present the current forms of rural tourism in the Republic of Serbia and to highlight opportunities for their development. The main hypothesis states that: „agritourism is the most common form of rural tourism in the Republic of Serbia”. In order to prove the thesis, a survey was realized from June 2012 until October 2013, involving 104 subjects of rural tourism on 11 districts (North Bačka, South Banat, Kolubara, Mačva, Moravički, Pirot, NorthBačka, Srem, Šumadija, West Bačka and Zlatibor) and 31 municipalities in Serbia. Due to the fact that in Serbia there are no official data on the total number of entities engaged in rural tourism, data from the National Association "Rural tourism of Serbia" were used. The sample was formed by random sampling. The instrument used in the study is the semi-structured questionnaire. The questionnaire was administered by interview. The main groups of questions were related to the characteristics of rural tourism operators and determining whether there is a link in the development of tourism and agriculture, as well as complementary services.

Sources of data in the completion of the survey are the subjects of rural tourism: rural tourism households, handicrafts, ethno houses, ethno villages, farmsteads, hotels that are located in rural areas, as well as other service providers in rural tourism in the Republic of Serbia. The research used quantitative methods - statistical method. In statistical analysis, survey research was obtained by the methods of descriptive statistical analysis. The main obstacle in the study authors had in securing sources of data due to the fact that in Serbia there is no register of rural tourism households. A database of the National Association "Rural tourism of Serbia" is the most comprehensive and authors used it. The study included 10.95% of the total number of providers of rural tourism in the Republic of Serbia, according to the specified database.

Results and discussion

According to the results of the survey, in the Republic of Serbia are represented the following forms of rural tourism: agritourism, farmsteads, event, wine, eco-rural, beekeeping and bio-agro tourism. Entities engaged in rural tourism and rural tourism forms which are not mentioned in the literature, can be called: agro-ornithological and eco-agro tourism.

Agritourism is, according to the results of statistical analysis, the most common form of rural tourism in Serbia. According to the results, the most of respondents (72.1%) are engaged in rural tourism and agriculture together, or in agritourism. These data prove the main hypothesis that the most common form of rural tourism in Serbia is agritourism (Table 1; Figure 1). The Example of good practice is a rural tourist household "Gostoljublje" in the village Mionica, Municipality of Kosjerić (Radović, 2015).

Table 1. Relationship between tourism and agriculture

Industries:	Frequency (number of providers)	Percentage (%)
– only tourism	21	20.2
– tourism and agriculture	75	72.1
– tourism and other industries	8	7.7
Total:	**104**	**100.0**

Source:Elaboration based on the questionnaire results

According to the results obtained by statistical analysis, it can be concluded that the agritourism is represented in all districts. This form of rural tourism is the most common in Zlatibor, Moravičkom, Kolubara, Šumadija, Mačva, Pirot and South Bačka District (Table 2).

According to the results of qualitative research, it can be concluded that the farmstead tourism, as a special form of rural tourism, is characteristic for the area of the Autonomous Province of Vojvodina and is well developed, promoted and popular rural tourism product. On some farms are kept the classic Vojvodina's way of life, and many were renovated and adapted to the needs of modern tourists, and have swimming pools, sports fields, ponds and other recreational facilities (Pejanović et al., 2014). According survey results, the examples of good practice regarding the quality of rural tourism offer are: "Dida Hornjakov farm", near Sombor and Mother's and Flower farm in Palić. Event tourism is based on a number of ethnographic and gastronomy events that are implemented in rural areas. It is estimated that around 1,500 different touristevents are organized annually in the Republic of Serbia. Municipality with the highest number of ethnographic and gastronomy events that are held throughout the year in its territory, is Subotica - 15 ethnographic and gastronomy events. (Pejanović and Radović, 2012)

Wine tourism is a new form of rural tourism in the Republic of Serbia. Ministry of Economy of the Republic of Serbia initiated the project "Wine roads of Serbia," which aims to define a tourist offer of wine tourism. In the framework of this project nine wine routes are defined, which are located on the tourist map of Europe. Wine roads in the Republic of Serbia are: (a) wine route of Fruška Gora; (B) wine route of Vršac; (C) Palić wine route; (D) wine route of Smederevo; (E) wine route of Oplenac; (F) wine route of Negotin; (G) wine route of Knjaževac; (H) wine route of Župa; (I) wine route of Kosovo and Metohija (Radović and Pejanović, 2013). The motive in rural areas (in eco-rural tourism) is conected with visiting protected nature areas in order to observe or study rare plant and animal species. If the accommodation, which are located nearby, in rural tourist households is offered to tourists, such tourist offer can be called eco-agro-tourism due to the fact that it is the product of two forms of rural tourism: agritourism and eco-rural tourism. According to the results, good

example is Rokin farm, located in the area of the Special Nature Reserve "Lake Ludaš" (Radović, 2015).

Table 2. Connection between tourism and agriculture by district

District		Activity of the respondents			Total:
		Only tourism	Tourism and agriculture	Tourism and other industries	
North Bačka	Number	2	2	2	6
	%	1.9%	1.9%	1.9%	5.8%
West Bačka	Number	3	4	4	11
	%	2.9%	3.8%	3.8%	10.6%
South Bačka	Number	3	3	0	6
	%	2.9%	2.9%	0%	5.8%
South Banat	Number	0	7	0	7
	%	0%	6.7%	0%	6.7%
Srem	Number	4	1	0	5
	%	3.8%	1.0%	0%	4.8%
Mačva	Number	0	5	0	5
	%	0%	4.8%	0%	4.8%
Kolubara	Number	1	10	0	11
	%	1.0%	9.6%	0%	10.6%
Šumadija	Number	1	9	1	11
	%	1.0%	8.7%	1.0%	10.6%
Moravički	Number	1	10	0	11
	%	1.0%	9.6%	0%	10.6%
Zlatibor	Number	4	20	1	25
	%	3.8%	19.2%	1.0%	24.0%
Pirot	Number	2	4	0	6
	% u	1.9%	3.8%	0%	5.8%
Total:	Number	**21**	**75**	**8**	**104**
	%	**20.2%**	**72.1%**	**7.7%**	**100.0%**

Source: Elaboration based on the questionnaire results

According to the results, there are entities engaged in beekeeping, but also in rural tourism. Due to the fact that beekeeping can be one of primary interest for the arrival of tourists, beekeeping tourism can be considered a unique form of rural tourism. Bio-agri-tourism is a special form of rural tourism and has begun to develop in the Republic of Serbia. This term means the tourist industry within the households engaged in agriculture in the system of organic food production. The term "agro-ornithological" rural tourism does not exist in the literature. The existence of this type of rural tourism is confirmed by the results of the realized survey research. In the Republic of Serbia there are entities that, in addition to agricultural production, are dealing with rural tourism and a basic tourist facilities or motive for tourists is bird watching. Based on the results of the realized survey research, example of good practice – „Kuća na Orlovoj steni“ ("House at the Eagle Cliff") in the village Komarani in the municipality of Nova Varoš (Radović, 2015).

Conclusion

According to the results from the conducted survey, it can be concluded that the most common form of rural tourism in Serbia is agritourism, which verify the hypothesis. Most of the surveyed subjects (72.1%) is engaged in rural tourism and agriculture, which points to a high synergistic relationship between these activities in the Republic of Serbia. In order to develop agritourism in the Republic of Serbia, based on the example of the Republic of Slovenia, the operators of agritourism should offer a minimum of 30% of products from own agricultural production. This will enable the development of rural tourism, but also the agricultural development considering that the economic evaluation of agricultural production is the most appropriate implemented through rural tourism. Rural tourism is a "consumer" of agricultural products, which affects the intensification and stable development of agriculture. Revenues realized from the tourism sector could be invested in development and modernization of agricultural production.

In order to develop all forms of rural tourism in the Republic of Serbia investments are needed in: (a) the development of tourist facilities; (b) development of rural infrastruture; (c) the development and training of staff; (d) the development and promotion (e) the development of sales channels of the rural tourist products in the domestic and international tourism market.

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Review paper
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THE ROLE OF TOURISM IN FOSTERING RURAL DEVELOPMENT

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Abstract

Tourism is the main source of foreign exchange earnings in almost 38% of countries and one of the five export categories in 83% of all countries. With a total number of 1,135 million tourists and income of $ 1,245 billion realized in 2014 and the expected number of 1.6 billion tourists in 2020, tourism represents one of economically most perspective activities. Tourism can have an important role in promoting rural areas as ecological oases with specific cultural and historic heritage. Rural tourism is most often identified with the "holiday village", but it also covers all other tourist activities in rural areas. Favorable natural and demographic conditions are the basic resources for the development of rural tourism. Around 9o% of European Union (EU) territory is considered a rural area on which more than 56% of population resides, therefore the politics of rural development is of vital importance for the further development of EU. Starting from characteristics of rural areas and European rural policy, the authors point out significant touristic potential that these areas have. In the paper is also analyzes the role and importance of tourism in preserving and promoting rural areas and fostering rural development at whole. Those analyses are based on contemporary national and international relevant sources and apply adequate methodologies such as: methods of analysis and synthesis, methods of induction and deduction as well as comparative method. Authors emphasis that the main positive effects of rural tourism development are: increasing the employment of the local population, the additional income for rural households, the development of production and services, prevention of migration at the level of rural-urban, protection and preservation of natural and cultural heritage and integrated development of rural areas.

Keywords: rural areas, tourism, rural tourism, rural development.

Introduction

From the very beginning of European integration of the fifties and sixties, were detected in significant economic and social disparities in the development of individual member countries. Today, after more than half a century of the EU, structural problems and problems of regional disparities are major problems of the European economy and society as a whole. The difference between the richest and poorest regions of Europe is 1:8, indicating that regional differences in Europe are not only still existing, but they are also very strong.

The effects of regional development are particularly sensitive rural areas. Although in the period 2000-2006 about 14 billion euros (7% of total assets) invested in rural development of the EU, however, the percentage of unemployment, especially youth, is very high in rural areas is as high as 17.6%. Besides the problems of unemployment, rural areas have a large demographic decline and aging of the population strongly (17% of the population are those over 65 years). It is therefore encouraging the development of small and medium-sized cities is crucial to the survival of rural areas inhabited by 5,000 to 100,000 people or 21% of European populations. In order to equalize economic development in countries and regions in the Union is defined by a common regional policy. The aim of the policy of balanced regional development policy is to achieve a harmonious, balanced and sustainable development and

strengthening of economic and social cohesion of the community, which is a common policy of balanced regional development and the EU called “cohesion policy” (Premović, Arsić, 2010).

Material and methods

Tourism is the main source of foreign exchange earnings in almost 38% of countries and one of the five export categories in 83% of all countries. At the same time, tourism participates with 9% of world GDP and about 30% of world trade in services. With a total number of 1,135 million tourists and income of $ 1,245 billion realized in 2014 and the expected number of 1.6 billion tourists in 2020, tourism represents one of economically most perspective activities (According to: UNWTO, Tourism Highlights, 2014; UNWTO, World Tourism Baromenter, 2015).

Actual results and projected future tourism growth, indicating that the need for strategic management of the tourism sector, primarily in the direction of control of further tourism growth. Changes in the contemporary world environment had its reflection in the field of tourism. Thus, after a period of domination of mass tourism at the end of the last century, the beginning of XXI century was marked tourist trips aimed at new destinations. The most interesting destinations for modern tourists are rural tourism destinations with untouched nature and specific anthropogenic values.

Rural development is a policy area which is vital for the EU because more than 56% of the population living in rural areas and 91% of the territory. By definition, the European Commission, the rural region is a territorial unit to economic and social structure of diversified activities. This entity can include villages, small towns and regional centers.

According to the OECD typology of rural regions are divided into three groups:

First regions where **over 50%** of the population lives in rural communities - **rural regions,**

Second regions in which **15 to 50%** of the population lives in rural communities - **significantly rural regions and transition regions**,

Third regions in which **less than 15%** of the population lives in rural communities - **mostly urban regions** (Network for Rural Development-Action Plan 2011-2015, 2010: 13).

Applying the OECD typology of EU regions in the example, we get the data by which rural regions make up about 55% of EU territory, significantly rural regions about 37% and 8% of EU territory can be considered semi-urban regions.

Contemporary politics of European Union moves to the center of local development for Rural Development, emphasizing rural areas, regions, and their coordination and harmonization.

In order to solve the problems of rural areas of the EU as a function of their development has an important role concept of SARD - Sustainable Agriculture and Rural Development. SARD includes the management and conservation of natural resources and direction of technological and institutional changes to achieve and continually meeting the needs of present and future generations. Thus, sustainable agriculture and rural development will enable the conservation of soil, water, plant and animal resources, without compromising the environment, with the technical and technological development (environmental) is applicable, socially acceptable and economically viable.

Analyzes of the role and importance of tourism in preserving and promoting rural areas and fostering rural development particularly in the European Union are based on contemporary national and international theoretical knowledge and relevant sources. Methods of analysis and synthesis, methods of induction and deduction as well as comparative method are used to explain the rural tourism and the main positive effects of rural tourism development.

Results and discussion

Rural tourism is often identified as “holiday village”, but it also includes all other activities in rural areas. The favorable natural conditions are the basic demographic resources for rural tourism. Positive effects of a rural development are: to increase employment of local people, extra income for rural households, development of manufacturing and service industries, preventing migration of the rural-urban level, the protection and preservation of natural and cultural heritage and integrated development of rural areas (Premović et al., 2011).
Rural tourism is comprised of more than nineteen different forms of tourism, such as the following: agro tourism/ farm tourism, residential tourism, heritage tourism, gastronomy and wine tourism, hunting and fishing tourism, cultural tourism, religious tourism, nautical tourism, continental tourism, school and educational tourism, medical tourism, sports recreational tourism, adventure tourism, camping tourism, transit tourism, nature and earth tourism, ecotourism, mixed and other forms of tourism.

Various possibilities for the development of rural areas depend on a number of factors, such as the following:
geographical location and accessibility of transport (rural areas in the proximity of urban regions, major public roads, processing capacities and the market itself have some developmental advantages);
natural conditions and potentials (the altitude, climate, land, forest cover, biodiversity, water resources, mining wealth);
human and material resources endowment (the infrastructure, industrial capacities, workforce qualification and selection, development of public services, size and morphological characteristics of settlements) and
social capital and interaction (cultural characteristics, attitudes towards tradition and modernization, horizontal and vertical cooperation, etc.) (Popović et al., 2011).

Tourism can play a significant role in promoting the rural areas as a kind of ecological oasis with specific cultural and historical heritage.

Tourism in rural areas, i.e. rural tourism is defined as tourism which provides a “rural environment” for visitors, by offering a combination of natural, cultural and human experiences which have typically rural character. The essence of rural life refers to the immersion of visitors in authentic, original and fundamental experiences. Rural tourism is what brings the visitor back to nature; back to the roots, back to basics, and embraces the return to the origins and originality. It is comprised of a spectrum of activities and services organized by rural population (Holland et al., 2003).
Therefore, various rural areas have significant comparative possibilities for environmentally sustainable tourism development. The main characteristic of rural tourism is to provide the visitors with the personal contact, a direct experience of physical, cultural and social natural settings for the purpose of their getting to know and understand the way of life of the local community, its tradition and values, so that they are eventually enabled to participate in one of its activities (UNWTO, Tourism & Leisure Advisory Services, 2011).

Tourism in rural areas is based on principles of sustainability and offers elements of country environment, nature, as well as presenting traditional hospitality and the values of life of the local population. It is the contact with nature and the personal human contact with the local people which makes rural tourism so unique. Rural accommodation combines different forms of tourism that showcase rural life, art, culture and heritage in rural locations. International

trends suggest that Rural Tourism is becoming an increasingly broader concept and that the needs and expectations of domestic and international demand are becoming ever more sophisticated.
Rural tourism, therefore, combines many different aspects of experiencing, sharing and showcasing rural life. These rural experiences can be defined in terms of rural activities and accommodation experience. It is the combination of the given forms that shapes the essence of rural tourism (UNWTO, Tourism & Leisure Advisory Services, 2011).

The main prevailing tendencies in tourist demand, particularly in Europe, are the following:
psychological and physical refreshment of urban people by traveling through the areas which differ geographically (tourist travels from low land to mountains, from towns to villages, from land to islands, etc.),
trend towards the experience in connection with cultural and historical heritage and preserved nature, "green" movement or tourism which is also called alternative and responsible, "soft", "good" or "new" tourism refers not only to tourism in the country but to tourism in cities as well as the littoral regions. Primarily, it implies pure water, clean and safe sea, healthy food and unpolluted air, i.e. it is not mass tourism but tourism which is environment friendly,
"blue" or nautical movement, i.e. demand, (cruise ship excursions by using marines, ports and harbors),
demand for the places, events and experience with prominent identity, integrity and differences,
demand for village (rural) and agro tourism, which implies stays in different accommodation types (not only in rural households) and performing activities (such as: sport, adventure, challenges, art, manual work, etc.),
demand for the health, spiritual and mental renewal and the identity renewal – refers to the new forms of medical, i.e. spa tourism: demand for a better condition (recreational activities, sport and exercises, diets, fitness) along with wishing to have a better health by means of reducing the levels of stress (www.cenort.rs. Downloaded to the site: 26.12.2013. Document: Village tourism, paper: The key questions of the rural tourism development).

When we talk about rural areas and opportunities for the launch of the tourist traffic, we must bear in mind that not all rural areas participate priori that market with its "tourist" offer. It is necessary that rural areas to first meet certain criteria. The basic criteria for the rural areas are given in the following chart.

Chart 1. The basic criteria for the rural tourist areas (Vuković et al., 2010).

CRITERIA	EXPLANATION
1. The location of households in their natural environment, a village or small town	Less than 5000 inhabitants in villages/towns or in very typical/traditional settlements
2. The rural area with emphasized characteristics of traditional agriculture and the outstanding natural values	Outstanding natural values (natural park, etc.). Traditional agriculture excluded industry
3. Tourism is not the main or predominant activity or source of income in the surrounding area	The ratio of the number of tourist beds and residents in rural areas should not exceed 1:1
4. A high level of environmental protection, a quiet and peaceful location, without any noise or pollution	Acceptable odors and noise which are characteristic of traditional agricultural production
5. Authentic accommodation and environment	_
6. Hospitality	The host takes personal care of his guests (tourists)
7. Small capacity units	The upper limit capacity is 40 beds, if not legally designated or prescribed by internal standardization by members
8. Compliance with the legal criteria for evaluation	Compliance with the standards adapted to evaluate quality
9. Social sustainability in the context of multifunctional activities in rural areas	The application of the criteria defined by “Agenda 21” for tourism
10. Connection with the local community and traditional culture	Minimum integration activities within the communities in the region, guests have the opportunity to make contact with local realities if they want to
11. Local products and gastronomy	Available in the environment
12. Culture (folklore, handicrafts, customs, heritage,...)	Available in the environment
13. Excluding criteria: - urban and industrial locality and their surroundings - areas of extreme mass and developed tourism - noise, - pollution and likewise	-

There have been approximately a million of registered rural households, providing “Farm and Village Tourism” services, by means of which 1.5 to 3 million job places are provided. The European continent is considered to be the leader in rural tourism with approximately 200.000 registered providers in rural tourism services and more than 2.000.000 tourist beds, whereas the number of direct and indirect employees in the European rural tourism industry is approximately 500.000. It has been estimated that the accommodation in farmhouses, private

village houses, small family boarding houses and hotels generate direct annual tourism expenditure of approximately 12 billion euros.
Taking into consideration the emphasized multiplicative character of tourist economy, it has been estimated that tourism has an indirect influence upon the additional 14 billion euros, which are achieved by means of the related industries, due to the rural tourism. Therefore, the contribution of rural tourism in the achieved outcome of the European industry amounts to approximately € 26 billion annually.

Conclusion

Tourism represents one of economically most perspective activities in world. The most interesting destinations for modern tourists are rural areas with untouched nature and specific anthropogenic values. Considered from the perspective of the modern business environment, rural areas are not only rounded agricultural areas intended solely primary production, but represent areas with significant potential for tourism development through meeting the various needs, such as environmental protection, preservation of traditional values, recreation, leisure, sports, different cultural and historical content, etc.
Based on previously findings, it can be concluded that tourism with its socio-economic effects and links to numerous economic and non-economic activities can be a significant factor in the promotion of rural areas as a function of encouraging rural development in general.

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Review paper
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RED PEPPER PRODUCTION CHARACTERISTICS – COMPARATIVE ANALYSIS IN EU COUNTRIES AND SERBIA

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Abstract

This paper analyses the production of the red paper of some important producers from EU countries. The analysis included six countries: Bulgaria, Greece, Spain, France, Italy, and Hungary. The following production characteristics were analysed and statistically processed: area of production, yields and total yearly production. Nominated countries encompassed about 80% of total production of red pepper in EU.

This research included official statistical data from EUROSTAT for the period of 13 years, 2000-2012. Descriptive statistical analysis included accountings of: average and extremes values of characteristics, coefficients of variations, and change rate. Furthermore, the results of analysis have been compared with the same data from Serbia.

The results of analysis have showed that:

- There has been tendency of decreasing the areas of red pepper in all observed countries (except Greece). The largest areas of red pepper were in Spain (21,000 ha), and Italy (13,000 ha). The average red pepper area in Serbia in observed period was 19,200 ha.
- Opposite to production areas, yields of red pepper had tendency of increasing (except in Italy). The highest yields were in Spain (48t/ha), Hungary (38.7t/ha) and France (31.5 t/ha). Average yield of red pepper in Serbia was 7.9 t/ha.
- Some of observed EU countries had tendencies of increasing production (Greece, change rate 4.1%, Hungary, 0.51%, and Spain, 0.20%), while some had tendencies of decreasing production (Bulgaria, -9.66%, Italy, -5.25%, and France, -1.67%). The largest producer of red pepper in EU was Spain, with more than one million tons. Next was Italy (318,000t). Average yearly production in Serbia was 152,000 tons.

Key words: red pepper, production analysis, EU countries, Serbia

Introduction

In this paper, the important producers of the red pepper production from the EU were analysed and compared with the data from Serbia. The analysis included six EU countries: Bulgaria, Greece, Spain, France, Italy, and Hungary. The area of production, yields, and harvested production were analysed, and statistically processed. Nominated countries consisted 80% of total production of red pepper in EU.

There are many papers about descriptive statistical analysis of vegetables (Mutavdzic et. al., 2011; Novkovic et. al., (2011, 2012, 2012a, 2014).

The goal of this research was to analyze the red pepper production parameters of the main producing countries in EU, and see comparative position of Serbia.

Materials and methods

In this research the quantitative methods were implemented. Observed period of analyzed data was 2000-2012. The data source was official publication of the Institute of Statistics of Serbia, and official statistical data from EUROSTAT for EU countries. The descriptive analysis included: average and extremes values of characteristics, coefficients of variations,

and change rate. The results of analysis of EU countries have been compared with the same data from Serbia.

Results and Discussion

The main results of descriptive statistical analysis of harvested area of red pepper are presented in table 1.

Table 1. Basic characteristics of red pepper harvested area (2000-12)

Country	Average (000ha)	Interval of variation		Coefficient of variation (%)	Change rate (%)
		Minimum	Maximum		
Bulgaria	7.55	3.00	13.90	52.79	-12.23
Greece	3.89	3.50	4.30	5.18	0.82
Spain	21.02	17.40	23.70	11.41	-2.37
France	0.65	0.50	0.80	13.92	-2.76
Italy	12.92	9.00	15.20	14.16	-3.90
Hungary	4.46	2.00	6.90	38.73	-9.47
Serbia[1]	19.20	18.47	20.02	2,60	-0.55
Republic of Srpska[1]	2.3	2.17	2.46	4.00	-0.52
Vojvodina[1]	4.6	4.10	4.92	6.60	-1.97

[1] 2001-10

According to the data presented in the table 1, it can be concluded that there has been tendency of decreasing the areas of red pepper in all observed countries (except Greece). Serbia ranks second in harvested area of red pepper (after Spain), and before Italy, on the third place. What is also positive, is a small coefficient of variation, and parctically, stable harvested area.

The changes of harvested area in EU countries in the obsereved period are presented in graph 1.

The results of descriptive analysis of yields of red pepper are presented in table 2. Opposite to harvested area, yields of red pepper have tendency of increasing (except in Italy). The highest yields were in Spain, Hungary and France. Average yield of red pepper in Serbia was far from maximal yields in EU, but there was a positive tendency.

Graph 1 Red pepper harvested area (2000-12)

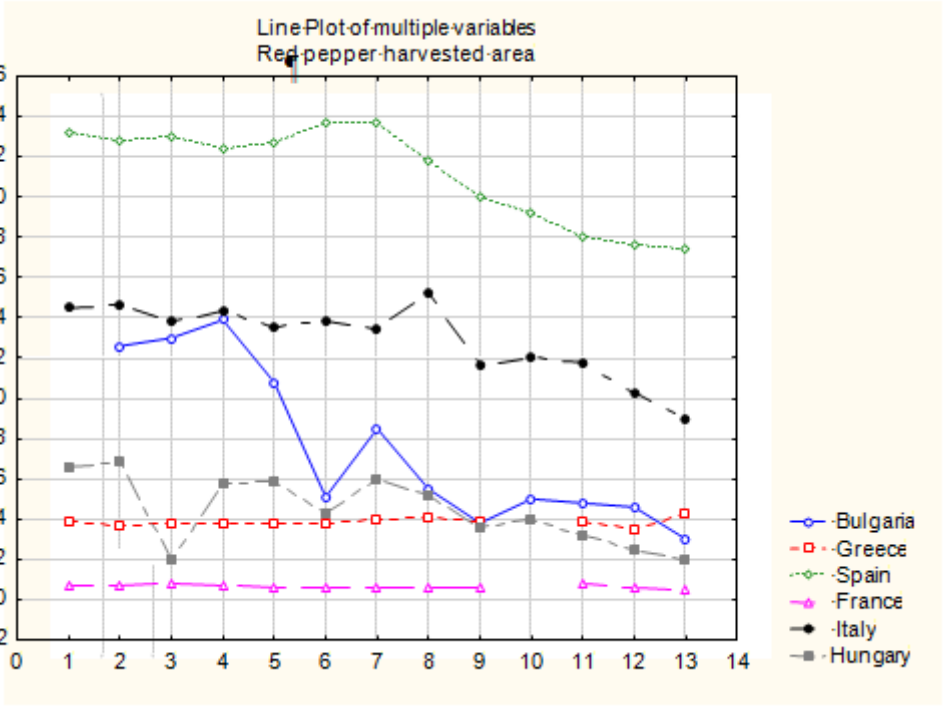


Table 2 Basic characteristics of red pepper yields (2000-12)

Country	Average (kg/ha)	Interval of variation		Coefficient of variation (%)	Change rate (%)
		Minimum	Maximum		
Bulgaria	14,419	11,430	18,400	13.30	2.93
Greece	29,353	21,130	39,410	19.29	3.33
Spain	48,049	40,810	55,630	8.38	2.62
France	31,509	10,100	42,170	26.05	1.68
Italy	24,449	19,060	27,840	10.15	-1.44
Hungary	38,709	18,900	72,000	45.93	11.03
Serbia[1]	7,900	6,800	9,200	10.31	2.32
Republic of Serpska[1]	9,900	5,800	12,200	21.07	7.90
Vojvodina[1]	11,053	9,130	14,339	18.62	5.01

[1] 2001-10

The changes of yield of red pepper in EU countries in the obsereved period are presented in graph 2.
The results of descriptive analysis of total year productions of red pepper are presented in table 3.
Some of observed EU countries have positive tendencies of total production (Greece, Hungary, and Spain), and some countries have tendencies of decreasing production (Bulgaria, Italy and France). The largest producer of red pepper in EU was Spain, with more than one million tons. Next was Italy with 1/3 production of Spain. Average yearly production in Serbia was half that of Italy.

Graph. 2 Red pepper yields (2000-12)

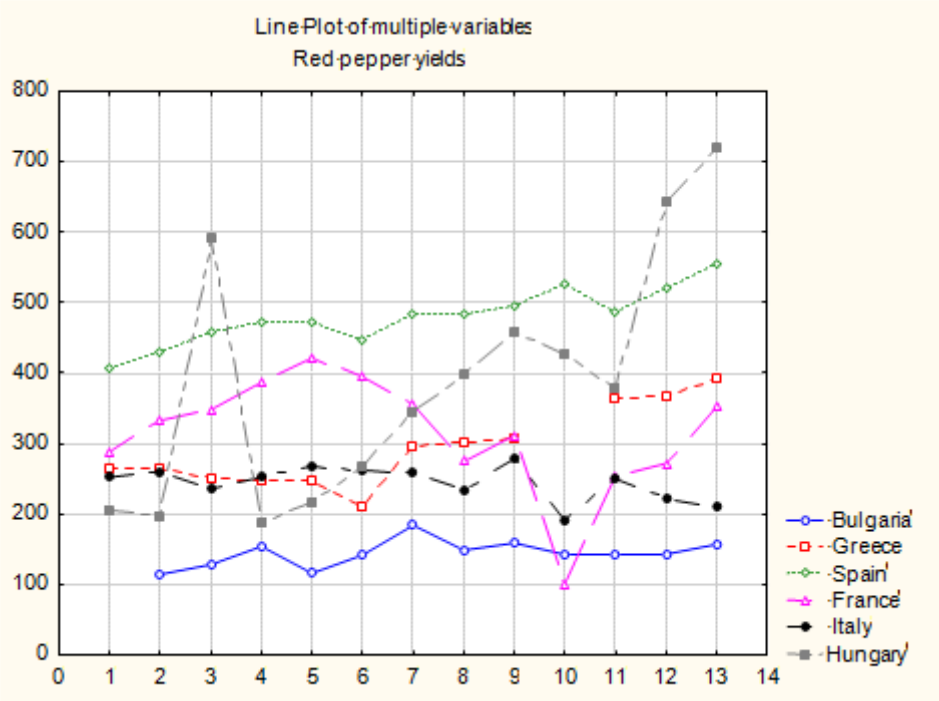


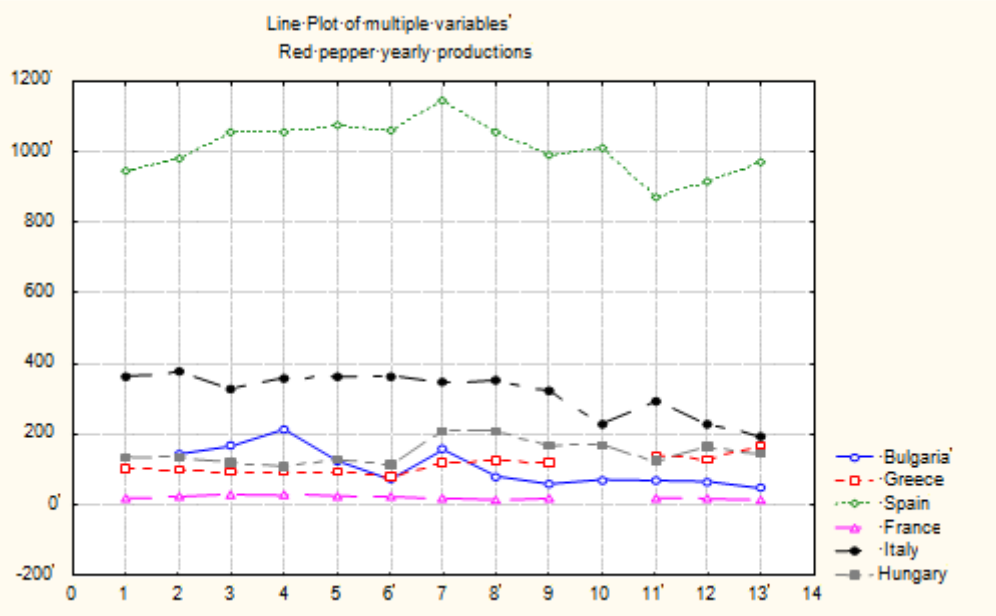
Table 3 Basic characteristics of red pepper yearly production (2000-12)

Country	Average (000t)	Interval of variation		Coefficient of variation (%)	Change rate (%)
		Minimum	Maximum		
Bulgaria	106.13	47.10	213.90	50.17	-9.66
Greece	113.98	80.30	167.90	21.60	4.10
Spain	1,011.20	873.00	1,147.80	7.35	0.20
France	21.43	16.50	27.80	18.64	-1.67
Italy	317.82	191.40	379.70	19.58	-5.25
Hungary	147.63	109.60	208.20	22.20	0.51
Serbia[1]	151.95	132.47	177.25	8.90	1.76
Republic of Serpska[1]	23.22	13.07	28.81	21.27	7.41
Vojvodina[1]	50.88	44.06	67.19	14.71	2.94

[1] 2001-10

The changes of year productions of red pepper in EU countries in the obsereved period are presented in graph 3.

Graph 3. Red pepper yearly productions (2000-12)



Conclusion

The results of analysis show that:

1. There is present tendency of decreasing the areas of red pepper in all observed countries (except Greece, where is present a slow increse). The largest areas of red pepper had Spain (about 21,000 ha), Italy (13,000 ha), and Bulgaria (7,500 ha).
2. Opposite of areas, yields of red pepper had tendency of incerease (except in Italy). The highest yields had Spain with 48 t/ha. Next were Hungary (39 t/ha) and France (32 t/ha).
3. Tendencies of total production depends of changes in area of production and yields. Tendency of increasing the production had only Greece. Spain showed tendency of stagnation, while in other countries were present tendency of decreasing. The largest producer of red pepper in EU was Spain, with about 1 million tons. The next were Italy (318,000 tons) and Hungary (148,000 tons).
4. Serbia ranks second in harvested area (19,200 ha), and had extremly small yield (7,900 kg/ha), which brough Serbia on the third place of red pepper producer in Europe (151,950 tons/year).

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GROSS MARGIN OF STRAWBERRY PRODUCTION IN POMORAVLJE REGION IN SERBIA

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Abstract

According to the Census of Agriculture 2012, the area under strawberries in Serbia amounted to 1.801 ha and the yield per unit area was 14,7 tons. The total production of strawberries varied from 24.910 tons in 2000 to 37.924 tons in 2008. The assortment of strawberries in Serbia is heterogeneous. New, high-quality varieties almost invariably prevail on farms close to large consumer centers. It is important for farmers to be introduced to the economic effect of a certain production line. The gross margin (GM) is a quick and efficient indicator for comparing production lines within different conditions. GM was used as an indicator of economic effects of production. Data on GM calculations were collected from a questionnaire survey conducted on a representative strawberry farm in Jagodina (Kovačevac village, Pomoravlje region, Serbia) in the period 2012–2014. For calculating the basic GM elements, the following data were used: data on yield and price; product price; quantity and cost of fertilizers, pesticides, and fuel; and costs of contracted services. GM does not indicate profits and, therefore, does not include the size and value of fixed costs. It represents the total value of production, subtracted by direct costs of investments (purchased inputs). Within the period 2012–2014, GM varied from 7.085,28 EUR/ha (2013) to 20.432,37 EUR/ha (2012). At the same time, total variable costs varied from 2.723,03 (2014) to 3.226,71 EUR/ha (2013). High yields in 2012 (33,33 tons/ha) and a substantially higher price led to an outstanding GM.

Key words: *gross margin, strawberry, variable costs, yield, price.*

Introducton

The gross margin (GM) is a quite useful tool for assessing the economic importance of a certain production line. Comprising solely the production value and variable costs, it can be easily comprehended even by people with no special training in economics. This is especially the case in production lines such as strawberry production, with one product as an output, and no additional by-products. In this case, the value of the entire production can be calculated simply by multiplying the quantity of product with its selling price per unit. Contracted services are not included in calculations, regarding the technology of this production. Moreover, one has to bear in mind that GMs of different production lines should not be compared if they have different overhead costs (Kuhlman et al., 2012).

GM should be considered as an important tool, since it gives the result calculated only in relation to what has been invested to produce the quantity in question, thus giving us the best overview, possible to trace over time. GM became widespread in the UK in the 1960s, being used for the first time by farm management advisors for making analyses and planning purposes (Barnard and Nix, 1979).

GM is a fast and efficient indicator of profitability of a certain production line (Filipović et al., 2015). In this paper, GM was used as an indicator narrower than an analytic calculation. The gross margin shows the difference between the cost of production and direct costs, which makes it an important tool from the economic aspect (Janković et al., 2009, Anđelić et al.,

2010, Tomić et al., 2013). In order to analyze economic results in agriculture and the structure of farms more easily, we used the methodology for calculation a standard GM as a scientific tool to support technical and economic orientation of farms (Iurchevici and Chetroiu, 2011).

Materials and Methods

Along with standard descriptive statistic methods, authors used GM calculations as an indicator of economic effects of strawberry production. Production data were collected from a questionnaire survey conducted on a representative farm in Pomoravlje region (Kovačevac village) during a 3-year period (2012–2014). Some basic elements for gross margin calculation were used: yield and price; quantity and value of fertilizers, pesticides, and fuel, as well as costs of contracted services. All prices are given in EUR, but original data were collected in Serbian dinars (RSD). Prices were calculated based on Serbian National Bank average exchange rate against major world currencies for particular year (www.nbs.rs/internet/cirilica/scripts/kl_prosecni.html). Indicators of the value of production, total variable costs and gross margin were calculated according to the methodology provided by the website of the Serbian Agriculture Advisory Service (www.psss.rs). The authors used *Microsoft Excel* for processing data and calculating GM, and the program was set to calculate the average value of each element of the calculation. GM is the difference of total revenue and total variable costs (TVC), achieved in a line of agricultural production per unit of production area (in crop production). We also calculated the critical values with the aim of estimating results of production under conditions of uncertainty. Critical values are those values at which the GM equates to zero (Subić et al., 2015).

Results and discussion

GM is adequate analytical basis, used for efficient cost management and decision-making. Also, the complexity of the production of strawberries imposes farmer need daily decision making crucial to maintain or increase the profit. The authors cannot assess the performance of the farm, based on the data on the farm size and the acreage of the used agricultural land. The analysis requires data on what, when and how much of something is produced, so that one can compare the performance of individual production lines and make decisions on the future structure of production. One of the derived indicators that can be used as a criterion for determining this structure is GM. GM is not the only and the most important analytical model, but it is a good starting point that requires relative simple data and methodology. GM represents the total value of production, subtracted by the direct (variable) costs (purchased inputs).

Table 1. Gross margin calculation, 2012

	Category	**Quantity**	**Price** (EURO)	**Amount** (EURO)
1.	Fresh strawberries	33.333,33 kg	0,707	23.572,18
A.	**TOTAL INCOME**			**23.572,18**
	Variable costs			
1.	Fertilizers			437,56
2.	Pesticides			362,20
3.	Fuel			453,90
4.	Labor			1.803,27
5.	Other supplies (packaging, string, ...)			123,75
6.	Contracted services			
B.	**TOTAL VARIABLE COSTS**			**3.180,70**
C.	**GROSS MARGIN (A – B)**			**20.391,48**
	Critical cost = 0,0954			
	Critical yield = 4.497,81			

Due to substantially high yield, both GM and critical cost in 2012 were significantely different than in 2013 and 2014 (GM was 3 times higher and critical cost twice lower). The most important variable cost was labor (1.803,27 EUR/ha), but this is quite expected, primarily having in mind manual harvesting of strawberries. Critical yield was 7,4 fold lower that achieved.

Table 2. Gross margin calculation, 2013

	Category	**Quantity**	**Price** (EURO)	**Amount** (EURO)
1.	Fresh strawberries	16.666,67 kg	0,619	10.312,83
A.	**TOTAL INCOME**			**10.312,83**
	Variable costs			
1.	Fertilizers			503,86
2.	Pesticides			431,46
3.	Fuel			523,74
4.	Labor			1.675,10
5.	Other supplies (packaging, string, ...)			141,43
6.	Contracted services			
B.	**TOTAL VARIABLE COSTS**			**3.226,97**
C.	**GROSS MARGIN (A – B)**			**7.085,86**
	Critical cost = 0,194			
	Critical yield = 5.215,14			

In 2013, lower yield was expected, but still significantly lower comparing to 2013. Additional lower price of strawberries led to almost 3 fold lower GM, while total variable costs were quite similar in both years. Labor costs were the most important in cost structure, as expected. Critical yield, compared to achieved yield was 3,2 fold lower.

Table 3. Gross margin calculation, 2014

	Category	**Quantity**	**Price** (EURO)	**Amount** (EURO)
1.	Fresh strawberries	19.444,44 kg	0,600	11.603,08
A.	**TOTAL INCOME**			**11.603,08**
	Variable costs			
1.	Fertilizers			409,19
2.	Pesticides			422,91
3.	Fuel			488,25
4.	Labor			1.332,84
5.	Other supplies (packaging, string, ...)			119,35
6.	Contracted services			
B.	**TOTAL VARIABLE COSTS**			**2.772,54**
C.	**GROSS MARGIN (A – B)**			**8.879,56**
	Critical cost = 0,143			
	Critical yield = 4.646,21			

GM in 2014 was high, having in mind that strawberry production in 3rd year is expected to be more stagnant, even higher than in 2013. Variable costs were similar as in two previous years. Again, labor costs were the highest one. Critical yield was 4,2 times lower than achieved.

Labor costs, as seen from annual GM tables, were the most dominant item in the variable cost structure (48,07 – 56,69%, 51,97 on the average), followed by group of costs with similar values: fuel (15,96%), fertilizers (14,63%) and pesticides (13,27%) (Table 4.).

Table 4. Structure of variable costs in strawberry production, 2012-2014

	Variable costs	Amount (EURO/ha)	(%)	Amount (EURO/ha)	(%)	Amount (EURO/ha)	(%)	Average %
		2012		2013		2014		2012-2014
1.	**Fertilizers**	**437,56**	**13,76**	**503,86**	**15,38**	**409,19**	**14,76**	**14,63**
	NPK	220,99	6,95	220,99	6,85	170,49	6,26	6,69
	KAN	176,79	5,56	176,79	5,48	119,35	4,38	5,14
	Foliar	39,78	1,25	106,07	3,29	119,35	4,38	2,97
2.	**Pesticides**	**362,20**	**11,39**	**431,46**	**13,17**	**422,91**	**15,25**	**13,27**
	Fungicides	182,76	5,75	197,21	6,11	191,89	7,05	6,30
	Insecticides	86,63	2,72	101,66	3,15	103,15	3,79	3,22
	Herbicides	92,82	2,92	132,59	4,11	127,87	4,70	3,91
3.	**Fuel**	**453,90**	**14,27**	**523,74**	**15,99**	**488,25**	**17,61**	**15,96**
	Crop cultivation	57,46	1,81	66,30	2,05	61,80	2,27	2,04
	Crop tending	28,73	0,90	33,15	1,03	30,90	1,13	1,02
	Crop protection	57,46	1,81	66,30	2,05	61,80	2,27	2,04
	Shredding crop residues	11,49	0,36	13,26	0,41	12,36	0,45	0,41
	Transport	114,91	3,61	132,59	4,11	123,61	4,54	4,09
	Irrigation	172,37	5,42	198,89	6,16	185,41	6,81	6,13
	Harvesting	11,49	0,36	13,26	0,41	12,36	0,45	0,41
4.	**Labor**	**1.803,27**	**56,69**	**1.675,10**	**51,14**	**1.332,84**	**48,07**	**51,97**
	Winter pruning	119,33	3,75	159,11	4,93	153,44	5,63	4,77
	Summer pruning	92,82	2,92	123,75	3,83	119,35	4,38	3,71
	Irrigation	0,00	0,00	66,30	2,05	37,08	1,36	1,14
	Harvesting	1.591,12	50,02	1.325,94	41,09	1.022,97	37,56	42,89
5.	**Other supplies** (packaging, strings, ...)	**123,75**	**3,89**	**141,43**	**4,32**	**119,35**	**4,30**	**4,17**
6.	**Contracted services**							
B.	**TOTAL VARIABLE COSTS**	**3.180,70**	**100**	**3.226,97**	**100**	**2.772,54**	**100**	**100**

The most dominant single item within TVC was labor cost for harvesting (42,89%), followed by costs for fertilizers (6,69 and 5,14%), fungicides (6,30%) and fuel for irrigation (6,13%). Generally, such range of cost items within TVC was expected, due to standard strawberry production practice requirements.

Conclusion

Within the period 2012-2014, GM varied from 7.085,28 EUR/ha (2013), to 20.432,37 EUR/ha (2012). At the same time, total variable costs ranged from 2.723,54 (2014) to 3.226,97 EUR/ha (2013). High yields in 2012 (33,33 tons/ha) and higher price led to significantly higher GM.

Within the variable costs, the cost of labor was the most dominant item (48–56%), but the final result given in GM of strawberry production substantially depends on yield and price. It is well known that yields are highest in the first year of production (2012), so higher income was expected. Anyway, a higher price in 2012 (0,1 EUR/kg, comparing to 2013 and 2014) made the difference in GM.

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AGRICULTURAL ADVISORY AND TRAINING IN SERBIA: THE CASE STUDY OF THE INSTITUTE FOR SCIENCE APPLICATION IN AGRICULTURE

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Abstract

Extension service in Serbia is well defined under the set of laws and regulation, covering all important aspects of advisory works such as service provision, training and licencing. It also authorises the Institute for Science Application in Agriculture (ISAA/IPN) to perform such activities. ISAA has been preparing annual training plans (ATPs) and implementing the trainings since 2010. In this paper we will analyse training period 2010-2011 and increase in advisors' knowledge in economic and advisory/extension skills. The training of advisors is structured upon 3 axes: knowledge increase - knowledge not covered by academic education; knowledge update - innovative technologies and practices in the field of agriculture; and advisory skills acquisition, i.e. applied knowledge, advisory techniques and 'soft' skills.

Agricultural advisors in the period from 2010 to 2015, within the IPN's annual training programme, have been educated through more than 40 different modules. Trainings organized by ISAA/IPN were attended by more than 14,000 agricultural experts: mostly extension agents, but also experts from farmers' associations, local government, Rural Development Centres, non-governmental organizations (NGOs) as well as farmers. Educations have been performed by eminent experts and professors from various universities from the USA and the European Union (EU), as well as experts from different Serbian faculties and institutes.

More than 80% of the trainees have responded that the trainings did meet their needs, making it very useful for them. Meanwhile 53% of the trainees have identified the "Farm management" module as a module with the greatest impact/importance to their work, followed by the "EU Integration" module and "Extension Skills". A minimum level of knowledge after the trainings has been assessed as 'good'. About a half of participants (49%) said they could fully participate on all the important issues during the trainings.

Keywords: *Agricultural extension, training, Farm management, skills, module, knowledge*

Introduction

To be successful in an increasingly competitive EU aligned market economy, farmers need to improve their overall performance. Technical efficiency to increase yields and product quality while optimizing use of inputs will enable reduce production costs. Marketing skills ensuring good prices and farm management will increase net margin and enhance farm profitability and competitiveness. Extension workers must be able to explain with simple words, why some actions must be performed from an agro-ecological point of view and why these actions influence yields or product quality. Eventually, to make farming competitive, producers will need sound advice in farm management (World Bank. 2007, Jankovic et al., 2013).

Once increase of income from primary agriculture production is achieved, farmer might still enhance their income by different strategies and will need additional advice. On top of the services needed to improve their competitiveness and income, farmers will have to comply with EU framework and adopt environmentally-friendly agriculture (Lampietti et al., 2009, van Berkum and Bogdanov, 2012.). Extension services should be able to provide assistance in the areas of Good Farming Practices and organic agriculture. Extension workers and advisors

must establish good relations with their customers - to respect farmers, take into account their knowledge, their problems and understand their needs. This attitude must be reflected in the extension practice. To this end, a range of extension techniques exists and extension workers have to be acquainted with them. The agricultural extension service in Serbia is expected to provide wide range of services including advices on production, market information, production economics at farm level, trade, standardization and protocols, plant protection, environmental protection and animal welfare, business planning, etc. (World Bank. 2007).
Before 2010, Agriculture Stations in Serbia were insufficiently adapted to the emerging changes and processes of transition. Advisors activities should be focused on creating opportunities in order to service commercial producers and shift from production-oriented to market oriented agriculture, from quantity production to quality production. The extension service in Serbia was however in 2007 primarily focusing on the provision of non-economic advice. Such system of agricultural advisory service was out of date in information and usefulness to emerging farming enterprise.
Since 2010 Extension service in Serbia is defined by Law and following sets of regulations. This framework covers all important aspects of advisory work (service provision, training, licencing). Institute for Science Application in Agriculture (ISAA) is authorised by law to perform trainings. Since 2010, ISAA has been preparing annual training plans (ATPs) and implementing the trainings. In this paper we will analyse training period 2010-2011.
The training of advisors is structured upon 3 axes: **knowledge increase** - knowledge not covered by academic education of advisors; **knowledge update** – covering innovative technologies and practices in the field of agriculture; and **advisory skills acquisition**, i.e. extension/ advisory skills, ‘soft’ skills and applied knowledge.
Agricultural advisors in the period from 2010 to 2015, within the IPN’s annual training programme, have been educated through more than 40 different modules. Trainings organized by IPN were attended by more than 14,000 agricultural experts: mostly extension agents, but also experts from farmers' associations, local government, Rural Development Centres, non-governmental organizations (NGOs) as well as farmers. Educations have been performed by top experts and professors from various universities from the USA and the European Union (EU), as well as experts from different Serbian faculties and institutes (World Bank, 2013).
The Objective of the paper is to analyse and assess increase in advisors’ economic (Farm management, European integration) knowledge and advisory/extension skills for training period 2010-2011.

Materials and Methods

Evaluation approach: As a part of the evaluation a number of surveys have been conducted and carried out among the training participants, in order to assess the results and effects they as beneficiary think they have gained. There were developed the questionnaires - Survey among project holders for the extension service – training.

Table 1. Number of questionnaires and response rate

	No of submitted questionnaires	No of received questionnaires	Response rate (%)	Statistic uncertainty	Delivery Method
Extension survey: training	277	150	54	+/- 5,4	Postal

Source: [World Bank, 2012]

Survey contained 10 background questions and five questions/ tables regarding results and effects of the trainings. Of those who replied to the survey, 90% (131) are employed by a public extension service, whereas only 15 respondents (10%) are working for a private extension provider. Also most of the respondents, 96% (144), are working full-time. 4% (6) are working part-time.
In addition, we used other standard methods for this type of research (desk research, a method of deduction, descriptive statistics).

Results

Training in 2010

In 2010 the first training modules were organized and carried out by Institute for Science Application in Agriculture (ISAA). Eight modules were implemented: 1.1: Farm management, 1.2: The EU integrations, 2.1: Modern technologies in crop and vegetable production, 2.2: Modern technologies in plant protection, 2.3: Modern technologies in fruit production, 2.4: Modern technologies in livestock production, 3.1: Extension skills 1, 3.2: Diagnostic research methodology and Farmers Need Assessment. In addition hereto a workshop on Monitoring and evaluating extension projects and extension agents' work was conducted as well. Twelve foreign and eight local consultants carried out the modules. 27 training courses with the total of 1.153 participants were conducted. The training modules included all extension agents from the agricultural extension service of Serbia (22) and the agricultural extension service of Vojvodina (12), 13 private extension services, three NGOs, two local administrations and one association.

Twelve representatives of the Agency for Agricultural Expert Services from Republic of Srpska (BH) attended the training courses on the EU integrations and 6 extension agents from the Extension Service of Montenegro and 2 extension agents from the Livestock selection service of Montenegro attended the training courses on modern technologies.

The US Department of Agriculture (USDA) also participated in the training courses. The cooperation in providing experienced trainers was first arranged for the module on Farm management, and then for the modules on Extension skills and Modern technologies in plant protection. The USDA also provided the trainers for the workshop on monitoring and evaluation.

Trainings in 2011

The 2011, the annual training plan (ATP) was focused on expanding and enhancing knowledge acquired through the 2010 Trainings, with an emphasis on developing practical tools and techniques that extension agents can use in the field. Based on the proposal of the ISAA, the following training plan for 2011 was approved and implemented:

Axis 1: Expanding knowledge modules:

1.3: Farm Management 2,
1.4: Farm Management 3,
1.5: Marketing,
1.6: FADN.

Axis 2: Updating knowledge modules:
2.5: Modern Crop and Vegetable Production Technologies 2, Application of Plant Nutrients in Crops and Vegetable Nutrition,
2.6: Modern Plant Protection Technologies 2, Integrated Pest Management (IPM) in Crop and Vegetable Production 2,
2.7: Modern Plant Protection Technologies 3, IPM - Crop and Vegetable Production 3,
2.8: Modern Technologies in Fruit Production 2, Application of Plant Nutrients in Pommes, Stone Fruit and Berries Production 2,
2.9: Modern Livestock Production Technologies 2, Modern Technologies in Animal Nutrition - Non-ruminants.

Axis 3: Applying knowledge modules:
3.3: Extension Skills 2 - Working With Written Media,
3.4: Extension Skills 3 - Working With Audio-Visual Media,
3.5: Farmers Need Assessment 2 - Acquiring skills for assessing the needs of farmers.

Within the period May until December 2011, ten training modules and testing of assistant extension agents were carried out, these were: 1.3: Farm Management 2, 1.6: FADN, 1.7. Nitrates Directive – Preparation of the Action Plan, 2.6: Modern Plant Protection Technologies 2 - IPM in Crop and Vegetable Production 2, 2.7: Modern Plant Protection Technologies 3 - IPM in Crop and Vegetable Production 3, 3.3: Extension Skills 2 - Working With Written Media, 3.5: Farmers Need Assessment 2- Acquiring skills for assessing farmers' needs in the new regions according to the module 3.3, 4.1: Training of Trainers - Extension Skills, 4.2: Training of Trainers – Agricultural Incentives, 4.3: Training of Trainers - Assessment of Farmers' Needs -Testing of the assistant extension agents for their admission to the Extension Service.
The training courses included extension agents from the agricultural extension service of Central Serbia (22 services) and Vojvodina (12 services), 13 private extension services, three NGOs, 2 local administrations and two farmers' associations, as well as 2,085 assistant extension agents. Also 2 extension agents from the Extension Service of Montenegro attended the training. In order to educate the 2,000 assistant extension agents in extension work methods and to make them qualified for direct work with the farmers as soon as possible, three educations were organized based on the *Training of Trainers* model.
Approximately 140 extension service agents - one or two from each of 34 Agricultural extension services stations - attended the training for one of the three modules: 4.1: Training of Trainers - Extension Skills, 4.2: Agricultural Incentives and 4.3: Assessment of Farmers' Needs. After the four-day training held at the ISAA, extension agents were enabled to educate future colleagues - assistant extension agents.

Relevance: It is obvious that the participants had gained a lot from participating in the training and that the training themes are considered to be relevant (table below). More than 80% (101) have responded that the training programme did meet the current training needs of their clients, thus making it useful for them.

Table 2: Did the training meet the current training needs of your clients?

	Percentage
Largely corresponding to	26% (32)
Corresponds to a certain extent	56% (69)
Corresponds to a small extent	16% (20)
Does not correspond	2% (2)
Don't know	1% (1)
Total	**100% (124)**

Source: World Bank, 2012.

Impacts: Participants at the training modules have experienced an increase in their knowledge and capacity in relation to “farm management” (table 3). Everybody had a “good” knowledge level as minimum after attending the training modules and the number of participants with an “excellent” knowledge has increased from 7% till 39%. This is considered to be a very positive result. Attending different training modules for majority of participants was a positive experience, mainly because of being involved in the discussions during the training course. 49% found that they remarkably (“excellent”) could take part in all the important issues during the training course.

Table 3: Self - assessment of Farm management knowledge[#]**:**

	BEFORE the training	**AFTER** the training?	Participation on all important matters **DURING** the training
Poor	7 % (9)	0 % (0)	0 % (0)
Relatively good	14 % (17)	0 % (0)	1 % (1)
Good	41 % (51)	11 % (14)	14 % (20)
Very good	28 % (35)	48 % (58)	36 % (51)
Excellent	7 % (9)	39 % (48)	49 % (69)
Don’t know	2 % (2)	2 % (2)	0 % (0)
Total	**100 % (123)**	**100 % (122)**	**100 % (141)**

#Scale from 1 to 5 where 1 = Poor, 5 = Excellent
Source: World Bank, 2012.

“The relationship between theory and practical application” during the training modules is assessed to be “good” to “very good” for most participants (71%), and 14% found the balance to be “excellent”. The rating of “usefulness of group exercises” is more positive, only 2% did find the utility of group exercises to be “poor”. The “length of the training” received a positive evaluation as well.

Table 4: Assessment of training content and performance[#]:

	The ratio between theory and practical application	The usefulness of group exercises	The duration of the training
Poor	6 % (9)	2 % (3)	3 % (5)
Relatively good	8 % (12)	6 % (9)	7 % (10)
Good	26 % (38)	25 % (37)	27 % (39)
Very good	45 % (65)	35 % (51)	39 % (56)
Excellent	14 % (20)	32 % (46)	24 % (34)
Don’t know	0 % (0)	0 % (0)	0 % (0)
Total	**100 % (144)**	**100 % (146)**	**100 % (144)**

#Scale from 1 to 5 where 1 = Poor, 5 = Excellent
Source: World Bank, 2012.

It is interesting to see that the “communication skills and abilities” have been improved significantly due to the trainings. 51% found their “communication skills” to be “excellent” after the training, which is an increase of 34% compared to their own baseline assessment.

Table 5: Self - assessment of communication skills[#]:

	BEFORE the training	**AFTER** the training	Rate the training as a whole
Poor	4 % (6)	0 % (0)	0 % (0)
Relatively good	10 % (15)	1 % (1)	5 % (7)
Good	28 % (40)	7 % (10)	16 % (23)
Very good	41 % (60)	41 % (59)	43 % (62)
Excellent	17 % (24)	52 % (75)	35 % (51)
Don’t know	0 % (0)	0 % (0)	1 % (2)
Total	**100 % (145)**	**100 % (145)**	**100 % (145)**

#Scale from 1 to 5 where 1 = Poor, 5 = Excellent
Source: World Bank, 2012.

Respectively 43% and 35% rated the training as a whole to be “very good” and “excellent”. Only very few of the respondents found that the training modules they have attended were not having any impacts on the factors listed below. On the contrary, the majority found that the training modules are contributing to: 1) improvement of the opportunities for the rural population to adapt to changes, 2) increase on focus and knowledge on farm management, 3) environmental protection and animal welfare, as well as 4) ecology and organic production.
In both years (2010 and 2011), most participants found the trainings to be very useful for their everyday work and to a large extent corresponded to the needs of their clients. The analysis of the tests in which participants anonymously rated their knowledge of key issues before and after each training course showed the improvement for all modules. Result from the training modules in 2011, ranging from 28% for the module 1.6 FADN to 38% for the module 1.7: Nitrates Directive- Preparation of an Action Plan.
The majority either „agree“ or „strongly agree“ that the trainings are contributing to increased focus and knowledge on sustainable agriculture, project work, getting through with ideas to the farmer and improvement the competitiveness of agriculture and understanding EU accession and IPARD.

Efficiency: 53% of the respondents found that the training module on “Farm management” had the greatest impact or importance to their work. This is followed by the “European Integration” (26%) and “Extension skills” (37%) as the third most important impact.

Table 6: Training with the greatest significance for work:

	The first response	The second response	The third response
Farm management	53 % (77)	0 % (0)	0 % (0)
European Integration	3 % (5)	26 % (18)	0 % (0)
FADN	0 % (0)	6 % (4)	20 % (7)
Modern technology: crop and vegetable production	10 % (14)	10 % (7)	11 % (4)
Modern technology: plant protection	8 % (11)	7 % (5)	0 % (0)
Modern technology: fruit production	5 % (7)	6 % (4)	9 % (3)
Modern technology: livestock production	4 % (6)	18 % (12)	6 % (2)
Extension skills	7 % (10)	21 % (14)	37 % (13)
Diagnostic studies and needs assessments	5 % (7)	6 % (4)	17 % (6)
Don’t know	6 % (9)	0 % (0)	0 % (0)
Total	**100 % (146)**	**100 % (68)**	**100 % (35)**

Source: World Bank, 2012.

Conclusion

Participants have gained a lot of knowledge from participating in the training, and training modules are considered to be relevant. More than 80% of the trainees have responded that the training did meet the current needs of their clients, thus making it very useful for them.
Participants have experienced an increase in their knowledge and capacity in relation to “Farm management”. Everybody has a “good” knowledge level as minimum after the training and the amount of participants with an “excellent” knowledge has increased from 7% till 39%. Majority of the participants had a positive experience in terms of being involved in the discussions during the trainings. The relationship between theory and practical application during the training is assessed to be “good” to “very good” for the majority of the participants (71%). “Farm management” module had the greatest impact or importance for their work (53%), followed by “European Integration” (26%) and “Extension skills” (37%). Feedback received during trainings confirms that the trainees are very much interested in acquiring knowledge and experiences. Training of advisors is generally needed to enhance the human capital and to facilitate the access to national support schemes, IPARD and other sources.

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EXPERIENCES IN ESTABLISHING THE SERBIAN FARM ACCOUNTANCY DATA NETWORK – FADN

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Abstract

In this paper we have given the experiences in establishing the Serbian Farm Accountancy Data Network – FADN by the project of technical assistance, financed by European Union through the pre-accession funds. This system represents the instrument for monitoring microeconomic indicators of farm performance and evaluation of effects of agricultural policies.

Objective of the work is to point out Serbian experiences in establishing FADN and possibilities for improvement and use of the system. Methodology used for writing this paper consists of data analysis and description of experiences of the authors, who participated in establishing Serbian FADN system. Besides that, comparison with experiences of other countries has been used, based on desk research and information obtained from our colleagues, FADN experts from EU member states.

Reasons for establishing FADN in Serbia, organizational structure established within the project period, data collection and data analysis methodology, as well as the methodology for analyzing first results are presented in this paper. Also presented are the expectations and needs that the system should meet in the future, in order to achieve sustainability and establish the necessary capacity to perform its main tasks.

Keywords: *FADN, Serbia, experience, capacities, methodology*.

Introduction

In the period of accession to the EU, Serbia is facing the great challenge of harmonizing the agricultural and rural development policy to the Common Agricultural Policy of the EU (CAP).

One of the tasks in this process is to establish the network of microeconomic data from agricultural holdings – FADN (Farm Accountancy Data Network).

This system represents the instrument for monitoring microeconomic indicators of farm performance and evaluation of effects of agricultural and rural development policies.

Serbia is in process of establishing the system through the project of technical assistance, financed by European Union through the pre-accession funds of the IPA instrument[********].

The project is delivered by the international consortium and is to be finalized by the beginning of October 2015. Main activities of the project consist of capacity building, training and education, development of methodology, development of software and database. Beneficiary institution is the Ministry of Agriculture and Environmental Protection (later referred to as Ministry).

[********] Instrument for Pre-accession Assistance

Materials and methods

Data on economic size of farms in Serbia from database of Statistical Office of the Republic of Serbia are analyzed and compared with adopted Serbian FADN selection plan, in order to determine representativeness of Serbian FADN sample. Experiences of the authors, who participated in establishing Serbian FADN system, as well as experiences of other countries, based on desk research and information obtained from FADN experts from EU member states have also been used in this paper.

Results and discussion

Institutional Structure and Capacities

In order to establish sustainable network, totally harmonized with FADN system in EU member states, Ministry decided to form organizational structure of the system proposed by the project team.

Ministry is responsible for Serbian FADN and future liaison agency is to be formed within the Ministry. Besides the Ministry, which is the central institution in Serbian FADN, two regional bodies are responsible for organization and coordination of data collection and data validation – Institute for Science Application in Agriculture (IPN) and Provincial Secretariat for Agriculture, Water Management and Forestry in Autonomous Province of Vojvodina (PSAWMF) with their responsible persons – regional coordinators.

IPN is coordinating body for agricultural extension service in Central Serbia (including Belgrade), while PSAWMF is responsible for extension offices in Vojvodina. Insufficient communication between these two is noticeable. Hence, more cooperation between these bodies is essential.

Besides these, other institutions were also involved in establishing the system during the project period: Agricultural Faculty of the University in Belgrade, Agricultural Faculty of the University in Novi Sad and the Statistical Office of the Republic of Serbia.

Steering committee was established to take a role of the FADN committee, after the project period. This committee consists of the representatives of all institutions that participate to the FADN in Serbia. Even so, so far the responsibilities of steering committee do not seem to be clearly defined.

Immediate data collection and communication with the farmers is delegated to the agricultural advisors who work at the Agricultural Extension Service. At the moment, there are 100 agricultural advisors working as FADN data collectors. Their everyday tasks, however, are very time-consuming which influences their job within FADN.

Ministry also has to fully apply FADN methodology, in order to establish the data network which is totally harmonized and comparable with EU member states.

Field of survey

The system monitors microeconomic indicators of farm businesses, but it does not represent all agricultural holdings in one member state. FADN field of observation consists of commercial farms. These farms gain most of their income from agriculture. Field of survey includes farms that cover at least 80% of total agricultural area and 80% of total output (of all farms). In case of Serbia this figure is about 203.000[††††††††]

FADN provides data on economic size of the farms and their share in total output. Since these information were not available so far from the FADN database, data from agricultural census 2012 were used for determination of the field of survey and designing the sample through the selection plan.

[††††††††] Source: Statistical Office of the Republic of Serbia

Table 1. Farms grouped by the economic size and their share in total output

	Number of farms	**Total standard output (EUR)**	**Share (%)**
100,000 or more	1,902	803,541,678	21.42
50,000-100,000	4,825	327,469,428	8.73
25,000-50,000	11,221	385,335,130	10.27
15,000-25,000	18,261	346,286,320	9.23
8,000-15,000	52,949	563,169,693	15.01
4,000-8,000	113,194	636,383,661	16.97
2,000-4,000	140,641	404,571,557	10.79
less than 2,000	288,559	284,033,429	7.57
TOTAL	**631,552**	**3,750,790,895**	100.00

Source: Statistical Office of the Republic of Serbia

Farms in Serbia in 2012 reached the standard output (SO) of 3,750 million EUR, which makes an average of about 5,939 EUR per farm. Threshold of 4000 EUR standard output was taken since the farms with more than 4000 EUR output represent 81.64 % of total standard output of all agricultural households in Serbia.[‡‡‡‡‡‡‡‡]

Sampling

Aimed sample for Serbia, before the accession, is 2000 agricultural holdings. This number of farms will make a representative sample for Serbian agricultural sector, meaning it will represent farms that cover more than 80% of total agricultural land and more than 80% of total farming output. Due to insufficient capacities, sample was built gradually. At the very beginning in 2012, *pilot sample* had only 40 farms. Later, during 2013 it grew up to 172 farms. In 2014 Serbian FADN has collected data from 500 farms for accounting year 2013.
For the first time, farms were chosen by selection plan, following statistical rules on sampling and weighting data. In 2015 Serbian FADN has already nearly 1000 farms with the data collected for accounting year 2014.
It is decided that Serbian FADN sample consists of two regions, which match the two NUTS 1 regions in Serbia – Serbia North and South. Serbia North consists of Autonomous Province of Vojvodina and Belgrade region, and Serbia South represents the rest of the territory[§§§§§§§§].
Data collection
Dataset for the FADN purposes is defined by Commission Implementing Regulation (EU) 2015/220 of 3 February 2015 laying down rules for the application of Council Regulation (EC) No 1217/2009 setting up a network for the collection of accountancy data on the incomes and business operation of agricultural holdings in the European Union.
Regulated set of all data collected from farm is called *farm return*. This set consists of: general information related to farm location, labor, type of production, economic size, data on assets and liabilities, detailed technical data related to yields and farming activities, detailed financial data on costs and income.
Besides data defined by the Regulation, member states may define additional set of data to be collected. In case of Serbia, these are information on rental state, owned land and quantities of all inputs.
Since there is no accountancy on most of the agricultural holdings in Serbia, project team, in cooperation with national and regional coordinating bodies, developed methodology for data

[‡‡‡‡‡‡‡‡] Source: Statistical Office of the Republic of Serbia
[§§§§§§§§] Due to specific political status, Kosovo is not included in Serbian FADN system.

collection from farms, both with and without accountancy. Data are collected directly from farmer by using questionnaire.

During the first year of the project (pilot project), data were collected for accounting year 2011, by visiting farms at the beginning of 2012 and recording all required information from 2011, by interviewing the farmers. Data were recorded by using simple questionnaire that was designed to contain all necessary information on farm return. In 2012, data were collected by using Questionnaire, filled by the data collector and the Log Book, where the farmer kept records on farm activities. Later, in 2013 and 2014, single document - Questionnaire was used directly on farm level, filled together by the farmer and the data collector.

Benefits for Farmers

During the project period, it was a great challenge to motivate farmers to participate to the system. Participation in the FADN is on a voluntary basis, and farmers in Serbia do not get any direct financial benefit from the system.

Therefore, it is essential to provide farmers with some direct benefit that will motivate them to join the FADN and to remain in the system. There are several direct benefits that farmers in Serbia may enjoy by participating in FADN:

1. Feedback to farmers in Serbia consists of four sets of information on farming business. Most of the information presented in this document are based on FADN standard results. The first set consists of general information, such as area, labor, yields, and land use. Next group of information are presented as a balance sheet. All farms assets and liabilities are grouped and presented here. This gives a basis for calculation of indicators on farms liquidity and solvency. Following group consists of financial indicators including profit and loss statement. This gives an overview and the structure on farm costs and income. Finally, the last set of information is called *Additional indicators*. Unlike, previous three groups, these indicators are not based on standard results. These indicators are totally decided by Serbian FADN project team, and are meant to provide relevant and useful information on farm business results to the farmers. For results of accounting year 2014, it has been decided that these indicators include: farm net income per ha, farm net income without subsidies per ha, farm net income per hour of unpaid labor, output/input ratio, debt ratio, liquidity ratio, gross margin of crops and products, gross margin of livestock and products, and gross margin of other products and services.
2. Even though in Serbia, farmers have valuable opportunity to use agricultural advisory services for free, Agricultural extension service employs about 230 agricultural advisors, which leads us to the number of nearly 3000 farms per one agricultural advisor. Since 2014 FADN is an integral part of the Annual Program of Agricultural Extension Service in Serbia, which makes FADN activities an obligatory task for a number of agricultural advisors[*********]. This means that the farmers who participate to the system have at least four visits of an advisor, just for FADN purposes. Advisors are (or become) familiar with the farming activities at these farms, and are able to give more detailed advises on improving farm business.
3. Naturally, at the beginning, most of the farmers kept records for FADN purposes with significant assistance from the data collectors (agricultural extension advisors). Later on they started to record their farm activities and the farm situation more on their own. This way, farmers start (or continue) with the good practice of keeping records at farm level, which is a good starting point for planning of future farm business, as well as a valuable input for business plans and other essential elements for different applications for loans or use of public funds.

[*********] At the moment, 100 agricultural advisors work within the FADN network as the data collectors.

Use of FADN data

During the project period the Ministry and other beneficiary institutions were provided with sufficient expertise regarding data analysis for policy making and scientific purposes.

Several workshops on this topic with national coordinating bodies, universities and institutes were organized.

Still, there is no clear idea for efficient utilization of FADN data for policy making purposes. Until now, no official analysis based on Serbian FADN data was published, neither the Ministry has issued any publication based on FADN data. This way there are no sufficient information on microeconomic indicators of Serbian agricultural sector (especially since the agricultural census in 2012). So far, it looks like that the Serbian FADN data is only planned to be used to fulfill the requirements of European Union, as a part of the accession process.

Conclusion

As the FADN system was introduced in 1965, lots of experiences were gained until now. The methodology is fully regulated by the EU, and it still develops, following the development of EU CAP. Also, experiences from countries that joined the EU not long ago (such as Croatia), are available to Serbian team.

Experience gained tells us that if the clear and stable institutional and organizational structure is established, Serbia has real chances to have a sustainable FADN system, harmonized with EU member states. Among other things, this means that the FADN staff should only work on FADN, since it is the only way for them to keep focus and energy for this serious job. Also, the Ministry and other involved institutions should use all available expertise and experiences from other countries, of which many faced the same challenges. Indispensable part of this process is a political will for establishing such a network, since the FADN is mainly a state-operated system.

In order to gain the interest of all possible stakeholders to FADN, possibilities for use of FADN data analysis and possible benefits for agricultural sector should be clearly presented any time possible, since the FADN data are valuable as much as we use them.

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THE GREEN INTEGRAL DEVELOPMENTAL MODEL CO-EVOLVED FROM GRASSROOTS INNOVATION MOVEMENT OF SLOVENIAN GOOD PRACTICES

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Abstract

Grassroots innovations are community-led solutions for sustainability that respond to the local situations, interests and values of the communities involved. Integral green Slovenia model is a holistic model of sustainable development, started as a bottom up initiative of a group of people from social responsible and sustainable institutions and SMEs representing already established or emerging good practices. They connected their stories to the theories of Integral Economics and Integral Development developed by Ronnie Lessem and Alexander Schieffer from Trans4m's Institute for Integral Development in Geneva (Switzerland), internationally recognized models of integral economy and sustainable development as a part of socially responsible practice. Nowadays it could be considered as a grassroots innovation movement in Slovenia that supports sustainable farming, preservation of vital rural areas, social inclusion, green industry, social entrepreneurship, sustainable tourism and self-sufficiency.
This paper presented the essential elements, history and evolution of the Integral green Slovenia initiative. The case study of Biotechnical Centre Naklo (Slovenia) was in detail analyzed since the uniqueness of its model and vision called CARE (Catalysation, Activism, Research and Education) that incorporates many innovative aspects of education for sustainable development (ESD), nature protection, organic farming, research and activism. An evident impact on local/regional community was achieved, but the ambition of this initiative is to inspire, connect and upgrade good practices in a creative way and impact on various EU developmental policies.
Keywords: *Sustainability, grassroots innovations, Integral green Slovenia initiative*

Introduction

Grassroots innovations could be described as networks of activists and organizations generating novel bottom up solutions for sustainable development. These solutions are adapted to the local natural and socio-cultural context, the interest and values of the communities involved (Seyfang and Smith, 2007). Indeed, grassroots innovations are driven by social needs and ideology. Therefore, aspects of social economies, mutual exchanges, voluntary inputs, social responsibility, ethics and values are included. A review of grassroots actions for sustainability (Church and Elster, 2002) identified a range of direct environmental benefits and significant socio-economic impacts with benefits for sustainable communities.
Transition theorist (Geels and Schot, 2007; Seyfang and Smith, 2007; Lawhon and Murphy, 2012) have made important contributions to our knowledge of the challenges and possibilities for achieving more sustainable societies. Potential development, needs and challenges faced by mentioned grassroots initiatives, are better understood if these community-level-activities are analysed as *innovative niches*. Niche situations are open spaces for new ideas, good practices and social movements that initially are not enough robust to challenge *socio-technical regimes* as described by transition theorists. Niches need to be transposed to wider scale. It could be done by policy interventions, which bridge niches and mainstream situations and reformulate wider scale practices. Green niches explore problems as mobility, food supply, energy and entrepreneurship and propose solutions as short food supply chains and

innovative local food systems, removable energy services, social entrepreneurship and shared-use mobility. But socio-technical regimes are barriers for radical changes, causing lock out of sustainable alternatives. To seed wider changes, drivers for change that are able to put pressure on regimes, are needed (Sterrenberg et al., 2014). Some examples of drivers are: climate changes, population growth, increasing oil prices and financial crisis. Altogether are powerful drivers that require a radical change in thinking of humans, some kind of transition thinking toward a more sustainable society that would lead us out of our comfort zone and nearer to sustainable outcomes.

Sustainability must be an integrated concept, but it is radically insufficient if it addresses just economic, social and environmental dimension without ethics, values, culture heritage and social responsibility. The integral green economy model by Lessem and Shieffer (2010) is based on four pillars which integrated the world's diversity of nature, community, economics, culture and spirituality, science and technology with the central ethical core. Moreover, CARE (C+A+R+E) model (Lessem et al., 2014) is a holistic approach that covers and connects activities of Catalysation, Activism, Research and Education. The collaboration between Alexander Schieffer and Ronnie Lessem, TRANS4M Center for Integral Development, Geneva, Switzerland, Darja Piciga, founder of the initiative for Integral Green Slovenia (Piciga, 2012) and BC Naklo started in May 2013.

Biotechnical Centre (BC) Naklo (2014, 2015) is a Slovenian public educational and research institution, ecologically orientated. In the Vision of the Centre is written that the Centre is a truly sustainable institution with a positive attitude towards nature, a concern for production and processing of healthy food and a great care for the natural environment in cooperation with the green economy sector. The Centre property (organic farm) covers 4 hectares of build-up area and extra 22 hectares of ecological agricultural area. It is divided in 3 main units: Secondary school, Higher Vocational College with Research Unit, Enterprise Centre. The Centre's major contribution to raising the awareness of local population about the environmental situation and conditions is based on an active approach, cultural and natural heritage protection actions and projects.

The aim of this article is to present the above described theoretical models co-evolved by Slovenian good practices as Integral green Slovenia movement, a bottom up designed grassroots innovative niche. The potential benefits and implications of shifting from the current economic and developmental models toward models of integral economy are discussed. The case study is focused on the green integral model of BC Naklo as one of the leading cases of good practices involved in the movement.

Materials and methods

In the article we propose a qualitative case study that is used to study complex phenomena within their context (Baxter and Jack, 2008). The case study methodology can be applied not only in the social sciences, but also on interdisciplinary, education, business and environmental studies. There are different interpretations of the notion of "case study" (Yin, 1994; Gillham, 2001; Baxter and Jack, 2008), but in general the case study should have a "case" which is the object of the study that should be complex and contemporary and should be investigated in its natural context with a multitude of methods (Johansson, 2003). In our case, the aim of the used approach is to present a model that could be implemented also in different environments and in consideration of local situations, natural and cultural background of the communities.

Results and discussion

The Integral Green Slovenia movement

The beginning of the Integral Green Slovenia movement can be traced back to 2011 when cooperation between its three leading figures - Darja Piciga, Ronny Lessem and Alexander Schieffer – started, but the idea of Slovenia's development as a model of integral, low carbon economy and society was presented for the first time in 2012 (Piciga, 2012; Piciga et al., 2015). The attempt of the strategy for the transition to a low-carbon society included key elements of Integral Worlds approach by Lessem and Schieffer (2010), based on four realms and the central moral core. It is a promising new paradigm for sustainable social and economic innovation on different levels (Bečić et al., 2013). This holistic model presented the vision of Slovenia as a highly integrated and inclusive society with an excellent business sector and a high quality of life, space and natural environment. The policy planning processes started in 2013 and led to the inclusion of the integral economy approach into the guidelines for action of some draft policy documents (Piciga, 2013). According to this approach, every social system needs to find, in order to be and stay sustainable, a dynamic balance between its four mutually reinforcing and interdependent realms, balanced by the central moral core. To analyse this model in the Slovenian environment (Figure 1), already existing good practices (representing institutions or enterprises that are leading cases of any realm) are used. The four realms co-evolved in Slovenia (Piciga et al., 2015) were: (a) the ‘southern’ natural and communal realm (promotes the self-sufficient community-based economy) represented by Šentrupert Municipality, (b) the ‘eastern’ cultural and spiritual realm (promotes a developmental culture-based economy) represented by BC Naklo (Vižintin, 2015) and The Heart of Slovenia (Gradišek, 2015), (c) the ‘northern’ scientific and technological realm (promotes a social knowledge based economy) represented by Domel, Železniki (Rejec et al., 2015), (d) the ‘western’ realm (revising as “living” life-based economic realm with a strong focus on ecology and sustainability) represented by Pipistrel, Ajdovščina. Indeed, the moral central core (related to the innermost value base founded in each society) was represented by Kindergarten Oton Župančič, Slovenska Bistrica (Leskovar et al., 2015).

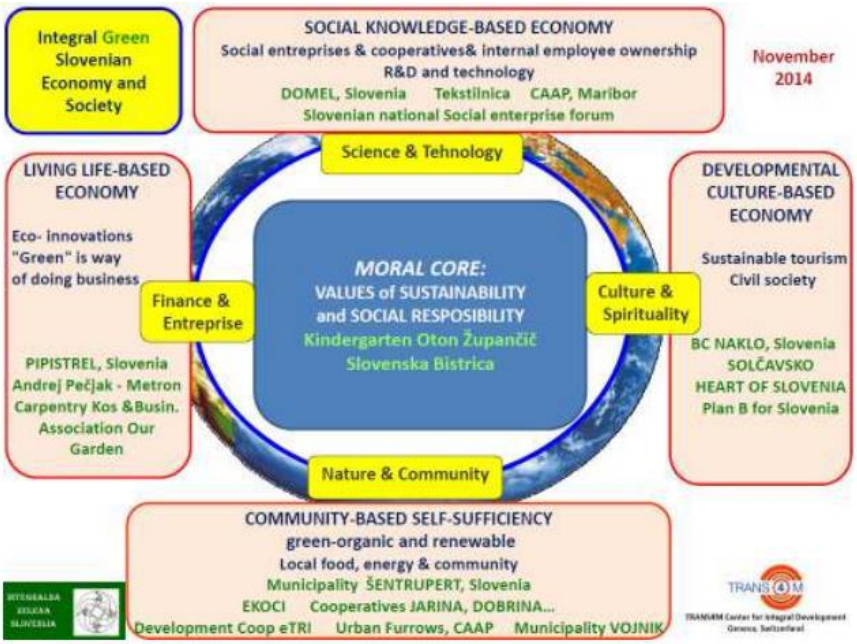

Figure 1: The model of Integral green Slovenia by Piciga et al. (2015).

The green integral model of BC Naklo

BC Naklo's adaptation called Green integral model of BC Naklo (Figure 2) is organised around a central ethical core defined through the positive social and environmental impact of the Centre (the emphases on the word "green" before "integral" is not casual). Great care is given to good interpersonal relationships, empowerment of the community, transparent administration, responsible entrepreneurship and the implementation of high-level certificated standards. Organic farming, low environmental impact of the estate, the use of renewable energy sources and ecological orientation are relevant indicators for the Centre's intense environmental engagement. The community of BC Naklo is growing continuously and has by now transcended national borders. Social responsible small and medium enterprises (SMEs) are supported by the Centre through joint efforts in education, production and services offered to the community and environment. Building on the integral green model, BC Naklo has set the following development and strategic objectives: (a) improving quality, (b) educating and training students and adult learners in the biotechnical field and related activities, (c) obtaining participants from the international environment, (d) becoming a Centre for sustainable rural development, (f) undertaking research and development activities to meet the needs of the economy at home and abroad, (g) implementing a wide range of projects, (h) development of green business ideas, (j) friendly working environment and employee development. To realize these purposes, the Centre has created good connection and solid collaboration with local authorities, communities, SMEs from green sector and non-governmental organizations (NGOs) in the fields of environmental protection, nature conservation and sustainable development. All these experiences of good collaboration practices are very important; together with values and cultural background they are the core of the success of the Centre. Nevertheless the Centre explores new ideas and innovative paths to give a more valuable contribution to strategies of local rural development and green economy. The goal is to obtain more relevant outcomes on the path of a sustainable lifestyle and responsible care for the environment.

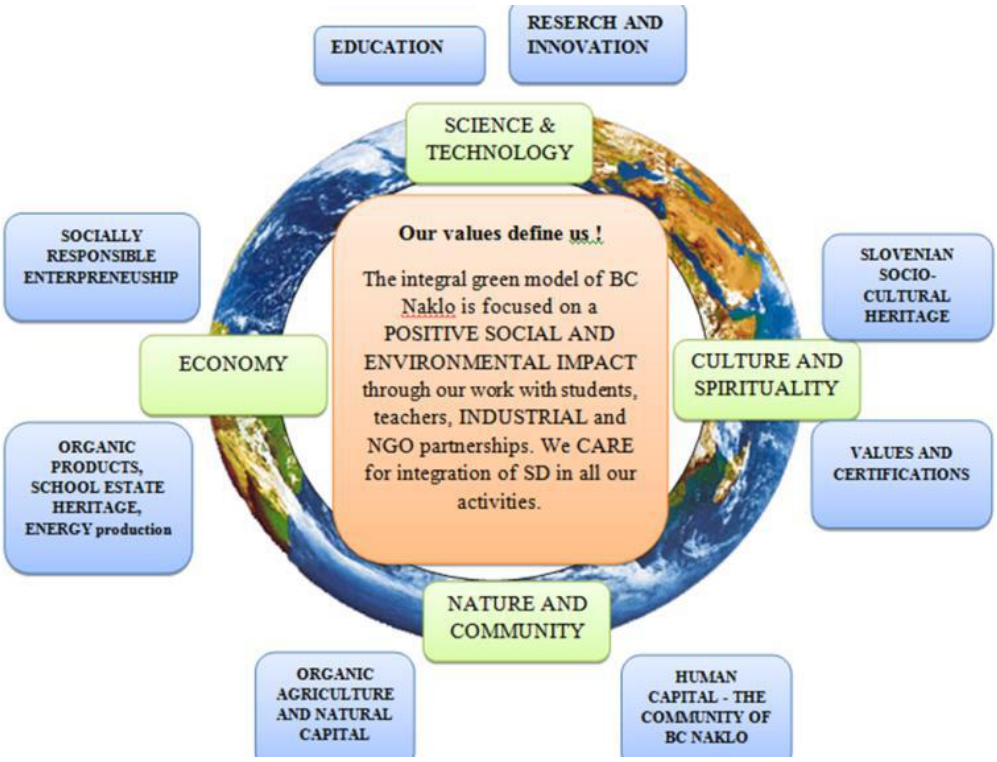

Figure 2: Green integral model of BC Naklo.

Central concepts as critical literacy, personal empowerment, mindfulness, human and nature well-being was promoted also by CARE (Catalysation + Activism + Research + Education) integration strategy that connect innovative education approaches like service learning and community-based-learning (CBL) with research, activism and the activation of consciousness of the community (Figure 3). The capacity for ethical reasoning present in human nature is the core of the future survival on this planet. Therefore new sustainable educational models are based on connection of nature protection, environmental and social sciences. CARE concept has different dimensions: (a) socio-cultural dimension that include caring for personal and professional development of students, employees and local community, (b) natural dimension that include caring for living organisms and for preservation of ecosystem dynamics and global ecosphere.

Figure 3: CARE model of BC Naklo (Modified from Lessem et al., 2014).

Regarding research, the integral framework for conceptualization was emphasized. Clarifying one's concepts with theory and practice (experiential learning) to understand the precise definitions could be a difficult step, but very useful since science aim to be explained and understood. It is a holistic approach that connects research and educational process to more affective domain (values, attitudes and behaviours). Indeed, “catalysation” is a process of activation of the consciousness of the community: we would like to create a community of BC Naklo that cultivates mindfulness of actions connected with protection of local cultural and natural capital. Mindfulness requires flexibility, openness, and the ability to break set (Hoy et al., 2006), which at the end could let to activism and pro-environmental behaviour. Environmental education that include CBL experiences as internships, volunteering, community service, out-of-the-class activities connects students’ theoretical work with their affective domain, values, interest and concern for the local community and nature. When students collaborate with local NGO, SMEs, organic farms and local community as activists, they are able to develop research projects, collect and analyse data, and share their results and conclusions, not just with their professors, but also with their outside tutors that can make use

of the information. The added values of these collaborations are much higher if outcomes are not just cognitive but also affective.

Conclusions

The theoretical framework of integral economics and development is being applied in an original and practical way in Slovenian natural and socio-cultural context. Starting from the moral core and introducing four economic paths (self-sufficient community-based economy, developmental culture-based economy, social knowledge based economy and “living” life-based economy) is an innovative and unique aspect of this approach. Slovenian integral green path is still considered as innovation niche that need to scale up. Evident impacts on local/regional community was already achieved, but the ambition of this initiative is to address global challenges, to develop sustainable economies and societies by inspiring, connecting and upgrading good practices at EU level and to weaving in a creative way various EU developmental policies.

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AGRICULTURAL EXTENSION AND ADVISORY SERVICES IN TUNISIA

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Abstract

Tunisian agriculture is facing many challenges such as climate change, new pests and diseases, food safety, export requirements, etc. Farmers find it difficult to get new knowledge to deal with these problems on their own. Therefore, extension services have an important role to play in supporting them. This study aims to analyse the contribution of the public extension services, to agricultural and rural development in Tunisia. For that semi-structured interviews were conducted in March-April 2015 with farmers and extension managers and agents in the central, regional and local levels. The analysis shows that Tunisian farmers have a variety of needs and priorities that exceed the traditional new agricultural technologies demand. Extension services face a lack of human resources and logistics. Gender has an important place in the national extension strategies whether in terms of woman share in extension staff or attention paid to woman farmers. Extension approaches are numerous and their evaluation is not well reported at the central level. Most of the extension techniques are old but some new ones are introduced in integrated rural development projects. That being said, it is recommended to provide more training to extension staff at all levels; to establish a rigorous planning of extension activities; to strengthen linkages with the research system; to adapt themes and messages to target groups and to introduce new extension approaches specifically for rural women. For making the extension system more efficient it is also necessary to foster farmers' organizations involvement in extension activities and financing and to improve extension infrastructure and equipment.

Keywords: *Agricultural extension, Rural development, Farmer organizations, Tunisia*

Introduction

In Tunisia agriculture sector is a fundamental source of economic growth. Despite the change and diversification observed in the Tunisian economy (industrialization, growth of service sector and expansion of tourism), the agricultural sector remains economically and socially important for its contribution to the achievement of national objectives as regards to food security, employment, regional equilibrium and social cohesion (Chebbi and Lachall, 2007). The importance of agriculture to the country's economy is highlighted also by its contribution to supporting rural livelihoods and controlling urban migration as it employs about a quarter of the Tunisian workforce (Touayi, 2004)

Over the last two decades, Tunisian agricultural growth can be largely attributed to the use of output-enhancing technologies and the promotion of investments in agricultural projects. For technological change, the advances have mainly consisted of the introduction of new seeds, new varieties, improved breeds, water saving techniques, and the mechanization of farming.

These advances have gone hand-in-hand with the promotion of investment projects in agriculture and credit facilities, and with more technically trained and educated farmers. However, improvements in the agricultural sector have not been uniform. In fact, many farmers are still poor because they lack capital, experience, and knowledge to become

efficient producers (Aoun, 2004). Agricultural extension is indeed considered as one of the tools contributing in the evolution of the agricultural sector and in the mobilisation of all its potentials so as to stimulate production and cover the trade balance deficit in food products (Touayi, 2004). Nowadays, Tunisian agriculture is facing other new challenges such as climate change, emerging and re-emerging pests and diseases, food safety, export requirements, etc. Farmers find it difficult to get new knowledge to deal with these problems on their own. Tunisian government is aware of these problems and tried to face them by providing the right equipment and the adequate quantity of inputs, but these means are not effective as long as farmers do not know how to use them effectively.
The present paper attempts to provide a critical analysis of the contribution of public agricultural extension and advisory services to agricultural and rural development in Tunisia.

Material and Methods

The methodology adopted is based on a review of secondary data and semi-structured interviews performed in March-April 2015 with a selected gender-balanced sample of agricultural extension and training providers and beneficiaries representing various structures dealing with public extension services. More particularly, the sample comprises Agriculture Ministry officers and administrative staff, farmers and private agricultural entrepreneurs, agricultural technicians, practitioners, representatives of NGOs, researchers and professors (Table 1). The output of these semi-structured interviews has been exploited to evaluate and show the qualitative aspects characterizing device performance as well as suggestions for improvement of the extension services in Tunisia.

Table 1. Sample used for semi-structured interviews and some information about the interviewees.

Number of interviewees	Occupation and/or Affiliation
3	Extension coordinators at central and regional level
3	Administrative officers in the Agricultural Development Regional Commissions
5	Heads of Extension territorial cells or Agricultural radiance cells
4	Regional representatives of farmers' associations and agricultural development groups' members
1	President of a society of agricultural services
2	Specialists from technical centres (citrus, potatoes and artichokes)
2	Researchers from National Institute of Field Crops and National Research Institute
2	Engineers from general directorates in the Ministry of Agriculture (agricultural production and plant protection)
1	Engineer from Agricultural extension and training agency
3	Agricultural inputs suppliers (1 society and 2 shops)
1	Professor in a high agricultural school
2	Former development projects' coordinators (one of them was in charge of rural woman extension)
4	Engineers from the Office of Livestock and Pasture, Office of Cereals, Office of Olive Oil, and Olive Tree Institute

Results and Discussion

Tunisian extension services framework

Successive reforms gave the current framework of the Tunisian public extension services. The results of these reforms is that the present public extension system is based on a single three-

level system (central, regional and local). In fact, reforms made during the 7th 5-year plan (1986-90) and 5-year national agricultural extension development initiative started in 1991 as part of the 8th 5-year plan (1990-94) have produced a three-tier extension system (MetaMeta, 2013): Agricultural Training and Extension Agency (AETA) at central level, Agricultural Development Regional Commissions (CRDA) at regional level, and Extension Territorial Cells (CTV) and Agricultural Radiance Centres (CRA) at local level.

At the *central level*, AETA is in charge of mass agricultural, extension, support, follow-up and coordination of field extension. According to the Law No. 90-73, AETA's missions relate to the areas of extension and agricultural training. As for strategy and management of extension systems and training, the Agency: contributes to the design and implementation of the national agricultural extension and training policy; ensures the development, monitoring and evaluation of training programs; undertakes actions and studies for the promotion of extension and agricultural training; coordinates extension systems implemented by the Regional commissions and ensures didactic and logistical support; produces and disseminates technical written and audio-visual material; helps farmers and their organizations to undertake extension activities. As for training, the Agency ensures professional training of farmers and extension agents retraining.

In the central level also, attached to the agriculture minister office, there is the Central Unit for Women's Promotion which is in charge of the extension for rural women and is represented at the regional level by the regional rural women promotion cell in each commission consisting of only women extension agents. Actually there is no recent statistics available about gender but the interviewees are satisfied with the presentation of women in the extension work. As an example in the Zaghouan regional commission the three coordinators of the regional extension cell are women. This is to show that gender equity has an important place in the national extension strategies whether in terms of woman share in extension staff or attention paid to woman farmer. However, a more participatory extension system would involve a greater representation of women (MetaMeta, 2013).

At the *regional level*, the Agricultural Development Regional Commissions are in charge of field agricultural extension through Territorial Extension Cells and the Agricultural Radiance Cells. In every commission, there are the directory for Extension and the Promotion of Agricultural Production and the coordination cell mainly in charge of planning, organisation and follow-up of agricultural extension actions and training; coordination with the different districts in the commission, technical services, and regional subject matter specialists who ensure support to agricultural extension activities in addition of reporting all.

At the *local level*, the Territorial Extension Cells are in charge of helping extension agents by organising their programmed work and providing means to them in addition of the follow-up. Agricultural Radiance Cells are in charge of: making new technology, information and options available to farmers; participating in the selection of research topics related to the field problems met by farmers; awareness raising and training of farmers to form groups (cooperative society, association); helping farmers' groups; etc. (Touay, 2004).

In general extension activity is carried out by the regional commissions (about 40%), public technical centres and offices (about 40%), professional structures (about 15%) and private advisory structures (about 5%). So the bigger part of agricultural extension and advisory activities is ensured by the public structures (about 80%).

National extension strategy

In the absence of an official document that sets the national extension strategy for the coming period, are presented hereafter the only documents available that address this issue, namely: the axes of the XIth Plan (2007-2011) in extension services; and the main development issues planned for 2010-14 period and which were summarized in a document prepared by AETA in

September 2010, few months before the revolution. This document is out of date, but gives an idea about the reflections made in the development of the agricultural extension sector.
The orientations of the *XIth Plan* (2007-11) in extension are mainly:
• Fostering initiatives towards bigger private sector participation, within a progressive program to the multiplicity of extension forms, based on: a public extension service for small farmers; a farmers' associations, organizations, cooperatives and groups extension for their members; a private extension for large farms and agricultural societies.
• Improving the effectiveness of intervention of AETA to follow technological progress;
• Deep thinking on the establishment of a new extension system that will be based on "*the mandate extension*".
In terms of extension approaches, the main recommendations are:
• The development of new approaches to extension as the tour the researchers during the cereals campaign in the regions visiting and advising farmers or famer-to-farmer extension or "farmer field schools"; which are used in some regions, but are not yet widespread. In other areas extension agents have never heard of such an approach.
• The development of strong relationship between research and extension;
• Improving the identification of extension needs by the involvement of the farmers' associations;
• Start a deep reflection on the establishment of a new extension system that will be based on "*the mandate extension*" like what was done with the "*sanitary mandate*" for animal health.

These orientations and recommendations are in line with the key focus areas of agricultural extension listed by the representatives of AETA at a workshop organised by FAO in 2003 (Touayi, 2004) namely: conception and implementation of a programming, follow-up and assessment approach; launching of *computerisation* in the extension system at the central and regional levels; and updating and enhancement of the programming approach by including the qualitative aspect (*programming by objective*) particularly in intensive extension campaigns.
As for the 2014 *strategy*, the main stated objectives were: strengthening the number of extension agents according to the goal of 1 agent for 600 farms; doubling the number of private consultants; encouraging development of new approaches to extension (e.g. Farmer Field Schools); providing more means to extension agents (e.g. transport, computers, internet connection); and capacity building of extension agents by retraining sessions.
The mass extension is nowadays one of the main activities of AETA and concerns the organization of radio and TV broadcasts, preparation of TV spots, printing brochures and posters, preparation of printed and electronic (i.e. CD, DVD) technical guides...
A systemic challenge for agricultural research, extension and planning institutions, whether government or non-government, is to institutionalize approaches and structures that encourage learning (Pretty, 1995). But nowadays in Tunisia, the perception of the current extension system by farmers and other actors varies from one to another, and is often negative. But overall, the majority of interviewees claim that the extension effort is getting weaker from one year to another, with decrease of extension agents in the field. According to MetaMeta (2013), Tunisian Agriculture extension is still to a large extent envisaged as a unidirectional process of transferring scientific knowledge to farmers without emphasis on farmers' traditional knowledge and practices, farmer-to-scientist or framer-to-farmer learning.
The creation of technical centres as links between research institutions and extension services was to well identify the research needs and disseminate research findings. It was expected the involvement of the farmers' associations, represented mainly by Tunisian Farming and Fishing Union, in the extension work by as a first step the preparation of annual extension programs but actually the involvement of the union is not effective. Many good attempts on extension are done by the Farmers' Syndicate, which is less important than the Farmers'

Union (e.g. information days on cereals crops). The material resources available to the extension agents of the Regional commissions are very poor. Thus in some extension cells, there is only one vehicle available for extension services (for both territorial extension cells and agricultural radiance cells attached to it). In addition, several vehicles dedicated to the extension are old (aged twenty years and more).
Touayi (2004) reported that in spite of the significant evolution in the agricultural extension sector in the past few years, agricultural extension faces many constraints, mainly: multiplicity of decision-making levels; number of topics to be extended is not proportional to the means made available to extension agents; lack of material and human resources at the central and regional levels (at the regional level, extension agents are called on to undertake many tasks beside agricultural extension activities); weak involvement of farmers and their organisations in extension system; and weak links between research and extension. Weak linkages between agricultural researchers, extension agents and farmers can be due also to the weak performance of the Agricultural Research and Higher Education Institute (IRESA) as one of the missions of IRESA is establishing linkages, on the one hand, between agricultural research and higher education institutions and, on the other hand, agricultural extension and farmers.

New technologies in extension

Regarding the use of the web and communication technology tools in extension almost all the territorial extension cells and agricultural radiance cells do not have Internet access or adequate didactic materials such as projectors, camera, etc. However, the National institute of field crops adopted since few years warning and exhortation mobile short messages from database that it has implemented and gathers farmers and extension agents. The messages concern irrigation based on the water deficit of each region, but also cereals disease. The majority of commissions, centres, general administrations, institutes, agencies have their own Facebook pages in which they publish information and sometimes in certain websites there are online software dealing with supporting farmers in some tasks like calculating irrigation amount. The AETA has a good database on its website with a lot of extension documents and videos available online.
A new information system for extension was set up. It provides for automatic processing of information and direct communication between AETA and the extension cells in the regions. For the implementation of this system, 11 governorates have been involved in the project, have been equipped with computer means and received specific training on the use of this system. But this system is out of use for the moment.
Audio-visual and printed material of AETA is un-optimized although it has its own audio-visual equipment used for the realization of extension materials. These devices are suitable for audio-visual production in accordance with professional standards, but AETA has a lack of internal skills required for such activity, which affects the ability of the agency to produce materials in sufficient quantity and quality.

Financing

The extension services are mostly public so the financing of extension projects comes mainly from public funds. The other structures dealing with extension, like farmers' organisations and some non-governmental organisations, are providing outputs and equipment (renting) in addition of extension free services or included in the final fees. Some of these societies have cooperation with external organization (*e.g.* IFAD - International Fund for Agricultural Development, etc.) or universities.
Budgets allocated for extension services in regional commissions are low and sometimes not effectively used. It is about of 50 thousand of Tunisian dinars (TD) (23000€) a year per

Regional commission, which means a budget from 5 (2300€) to 10 thousand of dinars (4600€) per territorial extension cell. Taking into account the many extension activities to be conducted by a territorial extension cell (information days, demonstration plots, visits to pilot farms, etc.), it turns out that such a budget is a major constraint for the implementation of the planned extension programs.

Examples of cooperation projects dealing with extension services

Integrated Agricultural Development Projects are carried out in some governorates and are controlled by the Regional commissions. We take here the example of two projects led in the governorates of Siliana and Kairouan.

The *Siliana's integrated project* lasted 10 years (2003-2013) aimed to improve the living conditions of local populations. The project is funded by IFAD, AFD (French Development Agency) and the GEF (Global Environment Fund). It includes an infrastructure component (development of irrigated area, water supply, rural roads, etc.) and an agricultural development component (tree plantations, creation of small and medium enterprise, etc.). Regarding extension, the project has planned an intervention by private extension among 500 farms spread over 17 sectors and over a period of 3 years.

Two private consultant societies were commissioned to carry out this project for a total budget of around 800,000 TD (350,000€). Each society has mobilized 4 or 5 extension agents that are supported when needed by experts or specialists in certain topics. Extension mission started by carrying out a participatory diagnostic with approximately 3000 farmers in order to identify their needs for extension and to choose later 500 to have the extension support. The choice of farmers was mainly related to the potential of development they can express and which fit with the objectives of agricultural development in the governorate (improve intensification and exploitation of irrigation, livestock development, etc.). It is expected that the 500 supported farmers will spread the knowledge to more or less 12000 surrounding farms. For each of 500 selected farms, a technical-economic profile was set up with the description of the starting situation (methods, tools, etc.) and performance (income, production, etc.), and the expected objectives at the end of the coaching period. Every three months an evaluation of the situation was held. According to the project team, the extension mandate experience was positive, since it has targeted farms in disadvantaged areas which were not well covered by the public extension services (because of lack of human and material resources), introduced into the region, participatory identification of extension needs approach and a method of defining objectives and evaluation of the impact of extension, succeeded in improving production in the majority of supported farms. This progress may be limited to the introduction of new techniques like the introduction of crop rotation and fodder crops, or the improvement of performance like lower livestock mortality rates and better yields.

The *Kairouan's integrated project* (8 years) was called "*Integrated Agricultural Development for Kairouan Hills*" covers more than 200000 ha (44% of the total area of arable land in the governorate) and includes 50% of the total population and 50000 households. The budget was 54 million dinars (25 million €), and includes an extension component. The project extension approach was an innovation in the region. In fact, the extension activity was subcontracted to private societies which recruited young engineers and planned for them complementary training according to specific project needs in the region. The contract with private societies was for 48 months and the total amount of compensation was around 1.2 million dinars (550 thousands €). A staff of 12 engineers, trained as "extension advisors", was divided into three teams called extension cells. Each cell is in charge of 250 to 400 farmers. In collaboration with the head of the Extension Territorial Cells concerned. It is expected that 5000 to 15000 farmers in the project area would benefit from good quality extension services.

The future of extension services in Tunisia: what role for private sector?

The private extension in Tunisia remains largely undeveloped and is limited either to specific interventions in big farms or some extension activities conducted under projects financed by public structures (AETA, Office of Livestock and Pasture, etc.) or external donors' funds. Thus, with the lack of a sustainable funding mechanism, private extension will remain very uncertain. It is expected that the farmers' associations, represented mainly by Tunisian Farming and Fishing Union, will be more associated to the extension efforts but actually the involvement of the union is not effective. National Agriculture and Fishing Union was occasionally involved in conducting extension activities through projects funded by donors. The aim was to test the transfer of the extension tasks from public structures to the farmers' associations. Upon completion of these projects, and given the absence of adequate resources (e.g. management, transport), many regional branch of National Agriculture and Fishing Union that were involved in these projects have had their extension activity annihilated and engineers recruited to extension stations are now managing administrative tasks. Other trials are led by some farmers' groups but the experiences ended with the end of projects.

Many good attempts on extension are done by the Farmers' Syndicate (*e.g.* information days on cereals crops).In addition, a successful agricultural services society provides extension, equipment and inputs for cereals' cultivators in a small area and the results are very encouraging. This syndicate has a cooperation with Emilia-Romagna Region in Italy.

Some pilot projects have been led with the "extension mandate" but these projects are few. *Extension mandate* was adopted as a sub-contracting approach by some organizations such as the Office of Livestock and Pasture and certain integrated rural development projects. These mandates are the subject of fixed-term contracts of around 3 to 5 years. The use of *extension mandate* remains, however, limited and the number of private consultants involved in projects would be about fifty.

One can mention as a successful experience that of the Office of Livestock and Pasture which use private advisors. Agreements have been signed with 22 private consultants to support groups of farmers in a 10 governorates (in the proportion of 1 to 3 consultants per governorate), over a period of 3 years. Funding for this program is provided by the budget of the Office. In addition to its positive impact on improving the technical knowledge and performance in livestock operations, this extension program has encouraged many private consultants to engage in this business. However, the sustainability of this program is not guaranteed in terms of financing, since there is no specific budget dedicated to it in the annual budget of the Office. With the absence of continued financing, the sustainability of private adviser jobs is not assured, and many consultants are looking to integrate the technical services of the Ministry of Agriculture.

The National Institute of Agronomic Studies, which is the most important and the oldest educational institution in Tunisia, has introduced a 5-year agricultural advisory diploma whose first promotion graduated in 2012 consisting of 40 persons (engineers in agricultural advisory). The first problem facing these young advisers was the fact that they have no experience in extension and that was a big constraint to be operational since the beginning.

According to the 2011 activity report of AETA, there are 177 private agricultural advisors but only a hundred are actually active.

The main obstacle to the development of this sector is the financing commonalities. Indeed, farmers, who are mostly small and the agricultural structures (Agricultural Development Groups, service societies, etc.) experiencing financial hardship are unable to use paid services of a private consultant. In addition to funding problems, other obstacles are to mention as competition with free extension agents as inputs suppliers, public extension, researchers and

lack of experience of some young private consultants, so that in some cases they could not win against the farmers who have extensive field experience.
However, private extension has several advantages compared to the public extension: flexibility and pragmatism in the contact with farmers, new extension tools, and looking for best results in efficient way and at the lowest price.
However, the farmers, especially small ones are not used to pay money for extension services. For this reasons, the private extension still very undeveloped and limited only for very specialized operations or complicated procedures or carried out under projects funded by public structures (e.g. AETA, Office of Livestock and Pasture) or foreign donors.

Conclusions

Tunisian agricultural extension is since many years in a continuous change and reforms to face new challenges. To support this dynamism, it is recommended to provide more training to public extension staff; to have extension activities better planned to be well supervised and improve efficiency; to strengthen linkages with the research institutions through contracts and agreements; to adapt themes and messages to target groups (including small-scale farmers) and to introduce new extension approaches specifically for rural women. For making the extension system more efficient it is also necessary to foster the involvement of other institutions in extension activities - like farmers' organizations and the private sector, which is largely undeveloped - get well planned budgets to be effectively used, optimize the extension resources in order to invest more in new technologies (*e.g.* ICTs) and to facilitate dissemination of research outputs/findings through more functional linkages between agricultural Research and Extension. It is also crucial to foster Public-Private Partnerships (PPP) related to extension work in order to create and strengthen synergies between the public and the private sectors. It is as well important to develop channels of knowledge transfer that facilitate learning from and among farmers.

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ADOPTION AND DIFFUSION OF GRASS SILAGE IN TRABZON PROVINCE OF TURKEY

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Abstract

Developments in agricultural sector largely depend on the application of advanced agricultural technologies. These technologies sometimes consist of very complex issues but sometimes include very simple practices. Use of these technologies especially in disadvantaged regions enables farmers to increase their income and welfare level, as well as, makes contributions to a reduction of developmental gaps among regions. Depending in the area where they are being used, the new technologies have one of the effects of decreasing production costs, increasing productivity, easing the work being done, and reducing labor requirement. Grass silage for small holder cattle owners is considered as one of these technologies for Trabzon Province of Turkey since it has not been commonly used in the region. The main objectives of this research were to determine socioeconomic characteristics and communication behavior of small holder cattle owners, to determine what practices are being applied by the farmers in the present farming system, to determine problems encountered by farmers, and to determine the awareness level of grass silage in the region and possible contributions that grass silage will make to the farmers in the region. It is aimed also to develop recommendations for the region to accelerate the adoption and diffusion process of this technology. Descriptive statistics were used for data analyses. Research findings showed that small holder cattle farmers are mostly older people and operate in conventional ways. They face serious problems with drying their grass and lack information about grass silage. In order to adopt grass silage, farmers must be aware of its benefits, and extension work is needed for this purpose.

Keywords: *grass silage, diffusion of innovations, adoption of innovations, Trabzon, Turkey*

Introduction

The use of advanced technology in agriculture, particularly in disadvantaged areas and regions make remarkable contributions to the income and welfare of farmers. Advanced technologies, depending on the area they are used, make it possible for farmers to reduce production costs, save labor, and play a role on increasing the quality and quantity of agricultural production. While contributing to the prosperity of the people of a region for the first time, any use of a technology can be considered as an innovation for the region. In this context, grass silage technology is an innovation for the Eastern Black Sea region, while the region is considered as a disadvantaged region due to a lack of sunny days during the harvest season. Grass silage is not a common technology that is being applied by farmers in the region. Even in Turkey, corn is the first crop that comes to mind in silage making. Although grass silage is used widely in developed countries, it is not common in Turkey, even in areas where the most intensive farming structures prevail. In order to understand the benefits of grass silage and promote it to the farmers of Trabzon province it is important to describe the existing unique farming systems of the region.

Trabzon is located on the Black Sea Coastal region of Turkey with a population of 766.782 and surface area of 4,685 square kilometers. It is bordered by the provinces of Rize from the east, Giresun from the west, and Gümüşhane and Bayburt from the south. The total area is

22.4% plateau and 77.6% hills (Wikipedia, 2015).

Agriculture is one of the main occupations providing livelihood to thousands of rural families in Trabzon. Majority of farms are specialized in hazelnut and tea production, particularly in coastal area. The eastern neighbor province, Rize is popular with tea plantations while the western neighbors of Giresun and Ordu provinces with hazelnut production in their coastal lands. Trabzon can to some extend economically grow both commodities. As we move to 40-50 kilometers inland from the coast the altitude goes up to 800-1200 meters and it becomes almost impossible to economically grow hazelnut and tea. However, geographical and climatic conditions in these altitudes allow farmers to grow maize, beans, potatoes, cabbage, and various fruits and vegetables. There are forests, pastures and meadows areas starting from these altitudes and going up to 2500 meters from sea level. Because the landscape is shaped by natural forces, it is almost impossible to use farm machinery in sloppy and fragmented parcels of lands, therefore farming activities largely depend of labor. These conditions create a different farming system producing mainly annual maize and beans in the operated fields and perennial grass for livestock. In addition, in the higher uphill and mountainous areas public rangelands are available for grazing livestock, mainly caws and sheep, from early June to late September, depending on the altitude.

Climate in Trabzon is considered suitable for livestock. Due to abundant rainfall grasslands (pasture and meadows) are always covered with lush grass. Particularly native breeds and Jersey for cattle, sheep and goats can graze at least 5 mounts in public rangelands. Grazing period can be extended to 8-10 months for sheep and goats but due to uphill and mountainous areas are covered by snow in the winter season, lower lands which are private properties must be utilized. During the winter season, hazelnut gardens can also provide grazing opportunities for sheep and goats. However, in order to utilize these lands for grazing purposes there must be an agreement between the land owner and the herd owner. Caws, on the other hand, kept in barns and fed by roughage and concentrate feeds which must be prepared from the summer season or purchased from feed marked. Many farmers have to purchase concentrate feeds but to be economically viable they must prepare their own roughage feeds from their own field. Therefore, they keep some part of grass land away of animal grazing and harvest it in early August for winter feeding.

Grass silage is expected to make two important contributions to animal husbandry in the region. The first one is it will provide sustainable roughage feed production which cannot be provided by the current farming system. This is because during the harvest season the weather is quite rainy in the research area and therefore, it is quite difficult- some seasons impossible because of continuing rainy days- to dry harvested grass for winter feeding. If grass is harvested in a sunny morning and it begins raining in the afternoon, grass cannot be dried. Some farmers collect and cover it by nylon until the sun appears again. But this time it is necessary to spread harvested grass in sun and let it completely dry. This procedure requires too much hard work and labor, and therefore, grass silage making will make another contribution to farmers which is labor save. In some years rainy days take so long that (a couple of weeks) and it becomes impossible for farmers to get dried grass form their own field. This lack of roughage feed results in selling the animals or purchasing roughage feed from other regions.

The primary purpose of this study was to investigate possible adoption and diffusion of grass silage making in Trabzon province of Turkey. The specific objectives were to determine socioeconomic characteristics and communication behavior of small holder cattle owners, to determine what practices are being applied by the farmers in the present farming system, to determine problems encountered by farmers, and to determine the awareness level of grass silage in the region and possible contributions that grass silage will make to the farmers in the region.

It is aimed also to develop recommendations for the region to accelerate the adoption and diffusion process of this technology. Research findings are intended to make contributions to the public in the region, researchers, extension personnel of the Ministry of Food Agriculture and Livestock, and other stakeholders.

Materials and Methods

Target population for this study was defined as small holder cattle owners in Trabzon province of Turkey. Two districts from this province, namely Çaykara and Maçka districts and three villages from each district (total six villages) were selected based on their agricultural potential, distinct from the city center, socioeconomic characteristics of rural communities, and potential of livestock production. From each village a list of small holder cattle owners showing their number of cattle was obtained from the District Agricultural Office. Lists of farmers and their cattle sizes from six selected villages made the accessible population of the study. Considering frequency distributions of the number of animals, accessible population was divided in three strata with 1-5 animals, 6-10 animals, and more than 10 animals. Yamane's (2001) stratified sample size determination formula was used to determine sample size. The equation for this formula is:

$$n = \frac{N\sum N_h S_h^2}{N^2 D^2 + \sum N_h S_h^2}$$

Where
n = sample size,
N = accessible population,
Nh = number of farmers in a stratum,
Sh = standard deviation within a stratum,
D^2 = desired variance,
e = accepted error from the mean, and
t = t value corresponding the accepted confidence interval.

Accepting 5 percent error from the mean (e) and 95 percent confidence interval (t = 1.645), the sample size was calculated as 52 farmers. This number was proportionally distributed to three strata, and respondents from each stratum were randomly selected.

Data were collected by conducting face to face interviews with farmers during which a questionnaire was administered. It included three sections. The first section included questions related to socioeconomic characteristics of respondents while the second section communication behaviors. Finally the third section included questions related to present farming systems in the region and possible adoption of grass silage making. Technically the questionnaire included both open ended and closed ended questions. Respondents' opinions and comments were noted for the open ended questions. Genever (2013), Budak et al., (2012), Güven (2011), Castro et al., (2010), Carvalho et al., (2010), Arslan and Dinç (2009), Bilal (2009), Bingöl et al., (2009), and Gebremedhin (2003) were utilized for preparing the questionnaire. Data were collected in March and April 2015. It was needed approximately half an hour to complete a questionnaire.

Descriptive statistics including frequencies, percentages, means, and standard deviations were used to analyze quantitative variables. For the items collected in a five point Likert scale, an interpretive scale was developed by the researchers. During data collection process many notes were taken by the researchers from respondents' comments in a given issue related to their farming system. If many farmers concerned about similar problems and made comments accordingly, these were also considered in data analysis process.

Results and discussion

Socioeconomic characteristics of respondents are presented in Table 1.

Table 1. Socioeconomic characteristics

Variable			Variable		
Age of farmer	n	%	Credit use for inputs	n	%
Younger than 35	5	9.6	Yes	17	32.7
35-50	15	28.8	No	35	67.3
Older than 50	32	61.5	TOTAL	52	100.0
TOTAL	52	100.0	Use of bank loans for investments		
Level of education			Yes	12	23.1
Illiterate	3	5.8	No	40	76.9
Elementary school	22	42.3	TOTAL	52	100.0
Secondary school	15	28.8	Yearly income		
High school	8	15.4	Less than 20 thousand	24	46.2
University	4	7.7	20-30 thousands	14	26.9
TOTAL	52	100.0	31-40 thousands	11	21.2
Cooperative membership			More than 40 thousands	3	5.8
Member	6	11.5	TOTAL	52	100.0
Not Member	46	88.5	Owning farm tractor		
TOTAL	52	100.0	Yes	0	0
Participation in village administration			No	52	100.0
Yes	12	23.1	TOTAL	52	100.0
No	40	76.9	Can you change your future by yourself?		
TOTAL	52	100.0	Yes	7	13.5
Amount of land			Somewhat	7	13.5
No land	6	11.5	No	38	73.1
Up to five decares	23	44.2	TOTAL	52	100.0
6-10 decares	10	19.2	Farming experience		
11-20 decares	5	9.6	Up to 10 years	6	11.5
More than 20 decares	8	15.4	11-20 years	7	13.5
TOTAL	52	100.0	21-30	9	17.3
Social security	N	%	More than 30	30	57.7
No social security	3	5.8	TOTAL	52	100.0
BAĞ-KUR	18	34.6	Income level		
SSK	30	57.6	Low	22	42.3
Green card	1	1.9	Medium	28	53.8
TOTAL	52	100.0	High	2	3.8
			TOTAL	52	100.0

Source: Author’s elaboration based on the questionnaire survey results.

From the table majority of respondents (61.5%) are older than 50 years of age, 43.3% are elementary school graduates, 88.5% aren’t cooperative members, 79.6% participated in village administration, 44.2% had land up to 5 decares, 94.2% had social security, 67.3% didn’t use credit for inputs, 76.9% didn’t use bank loans for investments, 46.2% had yearly income less than 20 thousand Turkish liras (1 US Dollars = 2.80 Turkish liras in August 14, 2015), no farmers had a farm tractor, 73.1% believed that they cannot change their future with their own effort, 57.7% had farming experience more than 30 years, and 53.3% considered themselves in the medium income category in their village.

Table 2. Communication behaviors

Variable	n	%	Variable	n	%
Reading newspaper			Travels to district center		
Every day	23	44.2	Every day	12	23.1
At least once a week	13	25.0	2-3- times a week	9	17.3
At least once a month	9	17.3	Once a week	22	42.3
Never	7	13.5	2-3 times a month	4	7.7
TOTAL	52	100.0	TOTAL	52	100.0
Listening to radio			Travels to province center		
2-3 hours a day	16	30.8	2-3 times a week	4	7.7
2-3 hours a week	7	13.5	Once a week	7	13.5
2-3 hours a month	4	7.7	2-3 times a month	15	28.8
Almost newer	25	48.1	Once a month	8	15.4
TOTAL	52	100.0	More seldom	18	34.6
Watching television			TOTAL	52	100.0
2-3 hours a day	50	96.2	Meeting with extension agents		
More seldom	2	3.8	2-3 times a week	4	7.6
TOTAL	52	100.0	Once a week	8	15.4
Awareness of the Internet			2-3 times a month	5	9.6
Yes	38	73.1	Almost never	35	67.3
No	14	26.9	TOTAL	52	100.0
TOTAL	52	100.0	Seeking farming advice		
Use of the Internet			Newer	5	9.6
2-3 hours a day	9	17.3	Always	22	42.3
2-3 hours a week	6	11.5	Sometimes	25	48.1
Newer	37	71.2	TOTAL	52	100.0
TOTAL	52	100.0	Taking part in farm events /seminars, field days etc.		
			Newer	40	76.9
			Many times	5	9.6
			A couple of times	7	13.5
			TOTAL	52	100.0

Source: Author’s elaboration based on the questionnaire survey results.

Communication behaviors of farmers are presented in Table 2. From the table it can be seen that 44.2% read newspaper every day. But during the interviews it was observed that most of the farmers read newspaper in tea-houses located in the center of the villages. Approximately half of the respondents listened to radio almost never while 96.2% watched television 2-3 hours a day. Approximately one-third of the respondents were aware of the internet but 71.2% almost newer used the Internet. Majority of the respondents traveled to district center at least once a week or more often while majority of the respondents traveled to province center at least once a month or more often. More than two-third of the respondent never met with extension personnel while almost half of them sought advise related to farming problems. More than three-fourth of the respondents never participated any farming events such as meeting, seminar, field day etc.

Table 3. Farming practices

Variable	n	%	Variable	n	%
Livestock provided			Meadows grazed properly		
Own production	49	94.2	Yes	40	76.9
Purchase	2	3.8	No	12	23.1
Own production+purchase	1	1.9	TOTAL	52	100.0
TOTAL	52	100.0	Manure used		
Dry grass provided			Own field	51	98.1
Own production /purchase	30	57.7	Wasted	1	1.9
Own production	13	25.0	TOTAL	52	100.0
Purchase	9	17.3	Change in number of animals recently		
TOTAL	52	100.0	Decrease	44	84.6
Concentrate feed provided			No change	8	15.4
Purchase	36	69.2	TOTAL	52	100.0
Own production /purchase	8	15.4			
Own production	8	15.4	% of grass properly dried		
TOTAL	52	100.0	0-40%	2	3.8
Livestock sold			%41-%60	14	26.9
Butchers	18	34.6	%61-%80	18	34.6
Ritual slaughter	15	28.8	%81-%100	18	34.6
No sales	14	26.9	TOTAL	52	100.0
Neighbors / market	5	9.6	Grass can be made silage without drying		
TOTAL	52	100.0	Yes	21	40.4
Observing animals for diseases			No	31	59.6
Yearly	27	51.9	TOTAL	52	100.0
Seasonal	23	44.2	Roughage concentrate feed ratio applied		
Never	2	3.8	Never	36	69.2
TOTAL	52	100.0	1/2 - ½	8	15.4
Type of barn	N	%	1/3 – 2/3	1	1.9
Concrete	38	71.1	1/4 - ¾	2	3.8
Wooden	14	26.9	2/3 – 1/3	5	9.6
TOTAL	52	100.0	TOTAL	52	100.0
Criteria of slaughtering time			Considering protein content of feeds		
Live weight	25	48.1	Never heart of it	41	78.8
Market price	3	5.8	No	11	21.2
Age of animal	24	46.2	TOTAL	52	100.0
TOTAL	52	100.0	Considering metabolic energy of feeds		
Source of information about diseases			Newer hearth of it	41	78.8
Extension service	4	7.7	No	11	21.1
Private veterinarians	48	92.3	TOTAL	52	100.0
TOTOAL	52	100.0			

Source: Author’s elaboration based on the questionnaire survey results.

Average small holder cattle farmer in the region owns 4.77 hybrid breeds, 1.21 native breeds, and 0.35 improved breeds. Selected farming practices applied by small holder cattle owners in the region are presented in Table 3. From the table 94.2% of the respondents provided their cattle by their own production, 57.7% provided dry grass from their own production, 69.2% provided concentrated feeds by purchasing from the market. Almost two-third of the respondents sold their cattle to butchers or for ritual slaughter every year. More than half of

the respondents yearly observed their cattle for diseases and other problems, almost half of the respondents considered live weight of the animals as a criteria when decided selling time, 92.3% used private veterinarians as information source about animal diseases, more than three-third reported that meadows were grazed properly, almost all respondent used manure in their own land, 84.6% experienced a decrease in their cattle size. Almost two-third of the respondents could properly dried 80% or less of their grass, 40.4% believed that grass can be turned in silage without drying, 69.9% didn't consider roughage to concentrated feed ratio in feeding their animals, 78.8% never heart of protein content of the feeds while the same percent of the respondents never heart of metabolic energy content of feeds. Research finding of this study showed that 84.6 percent of farmers reported that their number of cattle decreased (Table 3). Reasons for this decrease was investigated in a five point Likert scale from 1 = Not important at all, 2 = Not important, 3 = Neutral, 4 = Important, 5 = Very important. An interpretive scale considering the means was developed. According to this scale means were evaluated as 0.00-1.46 = Not important at all, 1.50-2.49 = Not important, 2.50-3.49 = Neutral, 3.50-4.49 = Important, and 4.50-5.00 = Very important. Considering this interpretive scale, the only item in the important category was lack of family members dealing with animals (Table 4). This was because young people in the region want to change occupation other than farming.

Providing roughage feeds is one of the most important problems in the area because of climatic conditions. Farmers were asked what kind of problems they encountered during providing their roughage feeds in a five point Likert scale and results are presented in Table 5. Considering the above interpretive scale drying of roughage feeds was the only item in the very important category. Harvesting, transportation, temporary storage in the field were in the important category. This finding verifies that providing roughage feed from own land is quite difficult in the region. Findings of this study showed that majority of farmers in the research area had no information about grass silage (Table 6). When the question "what can grass silage provide for you?" was asked on a five point Likert scale, respondents remained neutral in four of the five items and disagreed with the item "livestock would be better occupation". This finding showed that farmers lack information about grass silage and they need to be informed. They don't believe that grass silage will make livestock a better livelihood in the region.

Table 4. Reasons for decreasing cattle size

Awareness of grass silage	n	Mean	Standard deviation
Lack of family members dealing with animals	52	3.60	1.963
Feed prices high	52	3.23	1.967
Low income	52	3.19	2.010
Lack of governmental support	52	3.06	1.984
Lack of capital to purchase live animals	52	2.75	1.714
Illegal animal entries	52	2.42	1.913
Low meat prices	52	2.19	1.663
Lack of pasture and meadows	52	2.12	1.700
Low milk prices	52	1.71	1.446

Table 5. Problems encountered with roughage feeds

Problems encountered with roughage feeds	n	Mean	Standard deviation
Drying	52	4.62	0.599
Harvesting	52	4.08	1.186
Transportation	52	3.88	1.199
Temporary Storage in the field	52	3.87	1.299
Storage in the farm building	52	3.08	1.480

Table 6. Farmers' awareness of grass silage

Awareness of grass silage	n	%
I have never hearth about this practice	32	61.5
I have hearth it but have not enough information to apply it	10	19.8
I have heart it but my farm conditions do not provide adequate possibilities to practice it.	8	15.4
I have hearth it but I do not believe that it will be useful	2	3.8
TOTAL	52	100.0

Table 7. What grass silage can provide for farmers

What grass silage can provide	N	Mean	Standard deviation
Drying problem will disappear	52	2.90	2.070
It will provide more nutritious feeding	52	2.65	1.877
Reduces feed wastes	52	2.60	1.871
Labor save	52	2.50	1.863
Livestock would be better occupation	52	2.42	1.851

Conclusion

This study investigated the adoption and diffusion possibilities of grass silage among small holder cattle farmers in Trabzon province of Turkey. A socioeconomic profile of small holder cattle farmer in the region is an old man, elementary school graduate, has no membership in cooperatives, participated in village administration, own approximately 5 decares of land, had social security, doesn't use credit and bank loans, owns a yearly income about 20 thousand liras, doesn't have a tractor and has farming experience of more than 30 years. In terms of communication behavior he read newspaper mostly in tea-house of the village, doesn't listen radio too much but watch television every day. Although he is aware of the Internet he doesn't use it. He has regular travels to district and province centers but doesn't meet with extension personnel. Although he sometimes seeks farming advice from different people he doesn't participate in in farming events.

Farming in the region mostly carried out by conventional practices. Farmers are mostly older people and they have no option to switch profession. They must try to earn income as much as they can with their limited opportunities. Within the present farming system providing roughage feeds is quite problematic due to rainy season. It is also very much labor intensive. Grass silage will probably make it possible for farmers to produce cheaper and healthier died for livestock. This may increase the profit margins and attract younger farmers to stay in their villages.

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MARKETING COMMUNICATIONS IN PROCESSED AGRICULTURAL PRODUCTS: A CASE STUDY ON ÇUKUROVA REGION IN TURKEY

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Abstract

In this study, it is aimed to examine which level marketing communicating tools the businesses operating in the food industry in Çukurova Region, Turkey. For this purpose a survey was conducted with 92 enterprises in Adana and Mersin which operate in milk and dairy products, meat and meat products, fruit juice industry and 400 households living in Adana provinces determined by simple random sampling. As a result of the survey with enterprises; it was determined that they used the marketing communication tools (sales promotion, advertisement etc.) to increase sales, reduce inventory and so on, even though they aren't mostly aware of the concept of marketing communications and they indicate that they don't practice marketing communication programs. The mostly used tools by the enterprises are personal sales, direct marketing, sales promotions and advertisement. Also, as a result of the analysis, a correlation is determined between the enterprises' usage of marketing communication tools and brand and market performance. As a result of the survey with consumers, it is determined that the sales promotions are the most effective marketing communication tool when buying food products and changing the brand they use. When the consumers mainly buy food products they buy products which they decide in the market. In this case, advertisement, promotion and how natural the content of the product is the most important factors. Also it was determined that while buying different food products, consumers which have different gender, age, education and income levels, have different attitudes to marketing communication tools; such as young people mostly affected by media and primary and secondary school graduates mostly influenced by gifts.

Keywords: Marketing communication, marketing communication tools, consumer behaviour, food products, Çukurova Region.

Introduction

Economic and technological developments in the world create an intensively competitive environment. Particularly marketing with these developments, business structures, consumers and the buying behavior of consumers have changed. Today the changing customer profile, business information and communications technologies emphasizes the necessity to integrate to approach and strategies for sales and marketing (Babacan ve ark., 2008). Products on the market do not have very different properties with each other that is why there is an intense competition in the market. Since consumers prefer products of businesses from among these similar products, communication are becoming a major market power and businesses are forced to make changes to their marketing strategies. According to Mihart (2012), the survival of businesses in a competitive economic system and the development requires the existence of accurate and detailed information about customer behavior, motivation, the attitudes and behaviors. In order to meet consumer demand and their needs, enterprises should reveal appropriate products and services. To do this, they have to use appropriate marketing communication tools which are also help their profit (Uçak, 2004).

Marketing communications are a respond to their audience needs with changing existing business message and establishing a new communication channels with the creation

of new communication opportunities (Durmaz, 2001; Uçak, 2004; Ebren, 2006). Marketing communications are the means by which firms attempt to inform, persuade and remind consumers –directly or indirectly- about the products and brands they sell (Keller, 2001; Keller, 2009). A prerequisite for effective marketing communications is the consistency of sended messages through various channels of communications (Zavrsnik ve Jerman, 2011).

Marketing communications; to inform target consumers about products or services, if this is strengthening of their attitude and behavior in the desired direction, in the opposite direction change it or aims to create intended to a new attitude and behavior (Uçak, 2004; Durmaz, 2001; Ebren, 2006; Tuğrul, 2009). In the minds of consumers of messages transmitted with marketing communications to remain as the information and therefore, it is desirable to influence the purchasing decisions of future (Odabaşı ve Oyman, 2012; Uçak, 2004). Effective marketing communications contribute in brand awareness and the creation of a positive image (Erpelit, 2010). The instruments of marketing communications are regarded as advertising, sales promotion, public relations, direct marketing and personal selling (Jerman ve Zarvrsnik, 2013). Marketing communications; specifically in support promotion functions but in scope to, encompasses in all actions and components of the marketing (Özgül, 2008).

Consumers are having a hard time to choose between products and brands in the markets as their number is increasing and diversifying every day. In agricultural products, like others, various new brands are emerging to compete in market. So it is necessary to use marketing communication tools to affect consumer purchase behavior.

In this study, it is aimed to determine what percentage and how marketing communication tools that used by the food companies and its' affect on their business performance. Also marketing communication tools affect has been analyzed on consumer purchase behavior.

Materials and methods

Primary data for this study was obtained with face to face interview. The list of food companies in Adana and Mersin was gathered from Provincial Directorate of Food, Agriculture and Livestock. During the survey time 334 meat and meat products, milk and dairy products, fruit juice (soft drinks) companies were registered at Adana and Mersin Directorates (GTHB, 2014). Researchers are connected with all enterprises but only 92 companies from the population were willing to participate to the study and give information about their marketing communication tools. To select consumers "Based on Population Ratio Probability Simple Random Sampling" is used and calculated as 384 with 95% confidence interval and an error of 5% margin. However, 400 questionnaries were completed to prevent unusuable questionnaire. The questionnaire was related about consumer behavior in terms of choice of agro-industrial products.

Results and discussion

Out of 92 companies, milk and dairy products consist of 50%. Meat and meat products and fruit juice (beverage) companies consist of 29.35% and 20.65%, respectively. The average working time of the companies were 20 years and majority of them doing business at micro level. Companies of all three sectors were mainly trying to keep their position in business. Unfortunately, companies did not aware of the concept of marketing communications tools and their application in their companies. Juice companies implement marketing communications programs higher than others. In our study, it was found out that 79 company was classified as small, 12 company was medium and only one company was large. Mainly medium-sized companies had been applying marketing communications programs.

Although the vast majority of companies stated that they were unaware of the concept of marketing, it has been found out that many of them were using at least one marketing

communication tool (MCT) when they needed. The most widely used MCT by companies were direct selling, sales promotion, personal selling and ads. Fairs and sponsorship were the MCTs uncommon in these companies.

The tools used for advertising may have different effects on the consumers. The most widely used tool by companies for advertising were billboards and radio because of low cost. Esposito (2013), indicated that television is expensive and is considered not to be very effective to achieve specific goals. In this study it is found out that the companies do not use television as an advertisement tool because of high cost. Majority of companies (61.54%) used sale promotions such as advantageous packages, free trial, discounts and sales quota. Companies mainly used marketing communication tools to remind products (44.57%); to introduce new product (22.83%); and to maintain their position and to increase their sales (13.04%).

Scale, consisting of three dimensions prepared by Reid (2005), to measure brand performance -including performance related to sales, brand advantage, customer satisfaction- is used. In general enterprises assessed their level as intermediate in terms of brand performance. Correlation Analysis conducted in order to investigate whether a relationship between brand performance evaluation with marketing communication tools used. But Spearman were calculated because data did not normally distributed, in terms of communication tools variables. Spearman correlation coefficient was found 0.252 and p(0.015) which was lower than 0.05 that appears to be a linear relationship in a positive direction between brand performance evaluation with marketing communication tools used by companies. If the use of marketing communications tools are increased, also brand performance evaluation are also increased as a certain extent.

Changes in the specific topics of the last three years in order to measure the operating performance of the company were asked to evaluate. According to these assessments; companies stated that 52.17% had increased their market share, 56.52 % had increased of awareness gain on the market, 58.70% had increased to acquire of new customers, 51.09% had increased the consumer retention success and 51.09% has not unchanged the communication budget. Correlation analysis was performed to see whether there was a relationship between business performance with the use of marketing communications tools and whether there was a impact of brand performance on the market performance. Results indicated a positive linear relationship between the variables in both cases. This relationship showed that increasing use of marketing communication tools of company had increased of business performance and increasing the brand performance had increased market performance.

Multifactor analysis of variance was performed to investigate whether the companies showed a significant difference according to field of activity of business, company size and the type of company of brand performance, market performance and communication tools profitability impact assessments. When the data were analyzed, it was found that companies did not show a significant difference according to the business scope of the brand performance, market performance, communication tools profitability impact assessments ($p > 0.05$). In contrast, it was observed that no significant difference between the company size with market performance and communication tools profitability impact assessments profitability, which showed a significant difference between company size and brand performance ($p < 0.05$). In addition, it is observed that there was no found significant difference between types of companies with brand performance and market performance, which showed a significant difference between company type with communication tools profitability impact assessments profitability ($p < 0.05$).

According to the consumer survey results; respondents were 60% male and 40% women. The average age of consumers was 33 and 42% had a high school diploma and only

20% had a university degree. Majority of consumers were 36% craftsmen/artisans while 18% housewives, 14% workers/employees, 13% qualified experts, 11% students and 5% unemployed or retired. The average income was £ 1773 and about one-third of the income devoted to food expenditures. Food purchase was done mainly by mother (46.4%).

While consumers purchased food products and changed the brand they use, most affected tool of them was price reductions, the least affected the tool was advertising. It has been argued that this is because consumers often base their purchase decisions not on what they can explicitly remember about an advertisement but on what they know about the product (Zavrsnik ve Jerman, 2011). Roux and Zyl (2013) stated that more than 80% of consumer have a positive attitude towards sales promotional techniques and consumer have less disposable income were pleased from the extra products and low price that presented to them by companies using sales promotion techniques. Yılmaz et al. (2007), the vast majority of consumer groups (57%) reported that they received food according to advertisement. The research results showed that approximately 37% of the consumers had affected by advertisement before shopping, but the effect seems to be small compared to other tools. Although the effect while changing the brand was 29%, it is seen to be less effective than other tools. In granting purchase decision a product and replace new brand, sale promotions were more effective than other tools.

According to the research results, men had more affected from the sales efforts than women. Consumer behaviour was changing depend on gender. In this study, it was found that majority of men (63.01%) influenced by advertisement while women were most influenced by price reduction. While changing the brands that they used, the tool that the men were most influenced were free trial (61.03%), the tool that the women were most influenced were advantageous packages (42.05%). Overall, consumers who purchased products least affected from sales efforts were consumers aged 45 and above. Yılmaz et al. (2007), found that young people with 41% of group that most affected from the media. Our finding was also support this finding. When buying a certain product, younger consumers were also affected from other tools, which was advertisement. In changing of brand that used, while young group most affected from advertisement, other consumers were affected mainly from sale promotions. While literate, primary and secondary school graduates were influenced mostly by gifts buying a product, high school graduates were influenced by advertisement, college graduates were influenced by the price reductions. While product purchased, group who were least influenced from sales efforts were literate people. Consumers who have different levels of education had purchased products to influence from the different sales promotion techniques. While education level decreases, consumers seem to be more effected by tools such as gift, free trial. The group who had purchased products with the influence of marketing communication tools had income between £ 1000-1499. Consumers who had different income levels were affected from the different sales promotion techniques. Consumers with high income level had more affected from the ads when buying a particular product.

Product groups which purchase the most affected from advertisement were beverages (28.6%) and meat and meat products (25.1%). The product groups which purchase the most influenced from price reduction were milk and dairy products and meat and meat products. The product groups which purchase the most influenced from advantageous package were meat and meat products (45.9%) and beverages (45.1%). The product groups which purchase the most influenced from free trial were legumes (53.4%) and beverages (52.6%). The product groups which purchase the most influenced from gifts were beverages (46.6%), meat and meat products (43.4%). While the product groups which purchase the most influenced from the coupon were beverages (34.1%), the product groups which purchase the most influenced from awards were beverages (35.8%) and meat and meat products (32.3%). While consumers often purchased general food, milk and dairy products, meat and meat products, and the fruit

juices, they expressed not a particular brand that prefer. However, they spesifically pointed out the brands when it comes to milk and dairy products 43.1% and meat and meat products 42.1%.

When consumers purchased food products, the most important reason to prefer the brand was mainly reliability, taking into account the complaints and suggestions and ease of refund. Consumers were predominantly informed by seeing on sales area for the first time from products such as overall food (45.4%), milk and dairy products (43.1%), meat and meat products (41.1%) and fruit juices (40.9%). The product group which conducts mainly research were milk and dairy products and meat and meat products. Fruit juices were predominantly taken without any research. While the price was the most important reason when buying the product at the first time for general food (49.37%), meat and meat products (20:55%), fruit juices (18:55%) but the expiration date was important in milk and milk products (22.81%).

Decisions depend on advertisement, promotion and naturalness of the product had rated highly by consumers when it comes to milk and milk products. When buying the meat and meat products decisions were made mainly at the stores. When purchasing meat and meat products consumer were affected mainly by advertisement and promotions and the naturalness of the content was pointed out greatly important. While consumer purchased different product groups, Chi-square test was conducted to see whether a differences among attitudes toward marketing communications tools and among assessment of attitude against marketing communication tools according to product groups with consumer characteristics. There were differences among attitudes at 0.01 significance level ($p < 0.01$) while consumer purchased general foods, milk and dairy products, meat and meat products and juice. Consumers exhibited different attitudes toward marketing communication tools when buying different product groups. There are differences among attitudes at 0.01 significance level ($p < 0.01$) while consumer purchased general foods, milk and dairy products, meat and meat products and juice according to gender, age, education level, occupation and income. Consumers who had different features demonstrated different attitudes against marketing communication tools when buying different product groups.

Conclusion

Consequently, in order to create awareness for a product or brand in the consumers, informations of product or brand are required to present continuously to consumers of through variety communication tools because consumers mainly made their choices according to brand name coming to their mind when they have to choose among so many brands. In that sense, marketing communication tools are essential for creating awareness and ensuring the consistency of the messages delivered to consumers. In this study, although companies were not aware of the concept of marketing communications, marketing communications tools were used by one for specific purposes. While milk and milk products companies were mainly using direct sale, personal sale and sale promotions; meat and meat products companies were using direct sale and sale promotions and juice companies were mainly using marketing sale promotions and direct sale. With the use of marketing communication tools of businesses, there was a linear relationship between the brand and market performance. Also the companies were increasing market performance with increased brand's performance. Consumers were also affected by marketing communications tools when they purchased food products and replaced the brands they used.

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BERRY FRUIT GROWN AND MARKETED IN TURKEY

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Abstract

Berry fruits, which are quite liked and consumed much by consumers, can be assessed in variable ways. Antioxidants, minerals and vitamins included in berry fruits make them one of the indispensable choices of conscious consumers for healthy diet. Hence, berry fruits can be desired continuously in dmestic and foreign trade, and can be sold with high prices. Sale potential of berry fruits continuously increases more with the usage of these fruits along with in beverages, fruit yogurt/milk, dessert and consumption with alcohol more densely. Even though berry fruits have a great number of type and kind of commercial importance in the world, the vital berry fruit types in terms of commercial in Turkey are mulberries, strawberries, raspberries, and cranberries. According to data of Turkish Statistical Institute in 2014; 62.9 thousand tonnes of mulberries, 4.6 thousand tonnes of raspberries, 376.1 thousand tonnes of strawberries, and 10.9 thousand tonnes of cranberries were yielded in Turkey. In this study, evaluations will be made by analysing structure of berry fruits, which are commercially grown in Turkey, in terms of agricultural marketing components such as product, price, distribution and promotion. In this study, some researches, articles, presentations, thesis, and statistics were searched by considering relevant subject.

Keywords: *Mulberry, strawberry, raspberry, cranberry, agricultural marketing.*

Introduction

Berry fruits are significant agricultural products with respect to their relatively high demand elasticity against the change of their prices. Berry fruits are able to take place in the list of consumers in direct proportion to the recognition of their importance in terms of health. In other words, consumers demanding berry fruits are the ones having high health consciousness. Because the demand they have created allows these products to be sold in high prices (Ertürk and Geçer, 2012). Berry fruits are continuously demanded regarding domestic and foreign trade and can be sold with high prices (Onur, 1996). Sales potential of berry fruits increases in parallel to spread of fresh and processed product range. Sales potential of these fruits have increased more as a result of starting to be intensely used in production of beverages, fruit yogurt/milk, corn flakes, desert, and alcoholic drinks. This increase is also considerably supported with the image of health usefulness of berry fruits (Agaoglu, 2006). Within the rapid flow of modern life, women increasingly involved in business life and in parallel to the increase of consuming frozen foods have enhanced the importance of berry fruits which is source of these products. Additionally, another significant area of utilization of these fruits in both industry and homes is making jam and jelly. Due to the variation of berry fruits increased in terms of their use as fresh or frozen in food industry and especially their increasing importance in terms of dairy products industry, ice cream producers, confectioners and pastry cooks; these fruits are regularly processed in food sector as fresh, mashed, jam, molasses, syrup, or fruit juice (Yetgin, 2009). Big food producers use these products in large amounts. There is also a wide demand for berry fruits in alcoholic drink industry, as well. The fact that different natural herbal teas have become asked by consumers in recent years have brought the use of berry fruits into the forefront for this purpose

(Agaoglu, 2003). Types of cola and fizzy drinks constitute almost half of beverage industry abroad. Concentrated fruit juices constitute approximately the other half market of this amount. Share of berry fruits in this group is large. Rate of berry fruits increases within group of the fizzy drink. In this group; raspberry, strawberry, and black currants are regarded among products to increase the market share in future (Agaoglu, 2003).
According to data of Turkish statistical institute for the year 2014 in Turkey, total agricultural area is 23.943 thousand hectares and orchards with the rate of 13.52% constitute 3.237 thousand hectares of this. Rate of berry fruits among the total agricultural area is 2.15% and its rate among horticultural crops is 16.7%. Berry fruits constitute 27.73% of the total fruit production (Anonim, 2015a). For this reason, berry fruits are an agricultural activity with a different significance in economy of Turkey. They are also important because they allow assessing agricultural areas inappropriate to be utilized especially for growing other products (Yayar et al., 2006). In this study, assessments were performed by investigating the structure of marketing of berry fruits (mulberry, strawberry, raspberry and cranberry) in Turkey.

Raspberry

Since Turkey is among the native lands of raspberry, it has a profoundly suitable location in terms of raspberry cultivation. In addition to its consumption freshly; raspberry with its types of consumption regarding industry such as fruit juice, ice cream, pastry, deep freezing is on course of being a candidate fruit species to be an important cultivation branch in Turkey (Göktaş, 2011). Data have been started to be collected by Turkish Statistical Institute as from 1995 along with the establishment of commercial collective orchard lands for raspberry. Table 1 illustrates production areas, production amount, and yield levels for raspberry between 2004 and 2014. Production areas, being 3.5 thousand decares in 2004, started a downtrend trend beginning from this year. This downtrend has changed from 2011 towards the increasing trend. It was observed that raspberry production areas increased more than twice in 2012 compared to the previous year; despite a period of recession experienced in 2013, an increase of 4.14% was observed in 2014 compared to the previous year. Collective plant areas of raspberry increased to 4.8 thousands da in 2014 with an increase of 39.51% compared to 2004. Raspberry production, which was 2.2 tonnes in 2004, has shown a continuous increase in parallel with the increase of yield. By the influence of the increase in yield per decare up to 939 kg, a production of 4.6 thousand tonnes occurred in 2014 (Table 1)

Table 1. Production area, production amount, and yield of Raspberry (Anonymus, 2015a)

Year	Area (da)	Production (ton)	Mean yield (kg/da)
2004	3.500	2.200	629
2005	3.410	2.200	645
2006	3.387	1.997	590
2007	3.388	2.103	621
2008	3.397	2.050	603
2009	3.419	1.976	578
2010	2.198	1.980	901
2011	2.211	2.059	931
2012	4.675	4.080	873
2013	4.674	3.942	843
2014	4.883	4.587	939

Figure 1 illustrate 2003-based Agricultural Sector Producer Price Index and reformed Raspberry producer prices by months. According to this data, it was observed that producer prices were higher in June and July when Raspberry was relatively low in supply, lower in August when the product was abundant, and the prices started to increase by harvesting completely the product. Means of eight years indicated that the price showed stability in the rest periods of year. When the Figure 2 showing 2003-based Agricultural Sector Producer Price Index and corrected annual average take-home pays of the raspberry farmer between

2006-2014 was examined, it was observed that raspberry price being 3.46 TL/kg in 2006 fell to the lowest price level of the eight-year period by decreasing to 2.55 TL/kg in 2009 and raised to the highest price level of the same period by increasing to 4.39 TL/Kg in 2011. Producer prices of raspberry were 2.76 TL/Kg in 2012, 3.06 TL/Kg in 2013, and 2.91 TL/Kg in 2014. The fact that raspberry supply increase occurred in parallel with the increase in production areas and yield was higher than the demand for raspberry could be asserted to be effective in low level price formation in recent years.

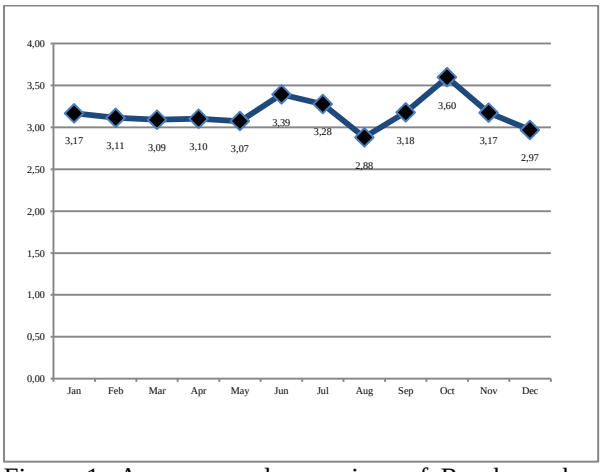

Figure 1. Average producer prices of Raspberry by months (2006-2014)

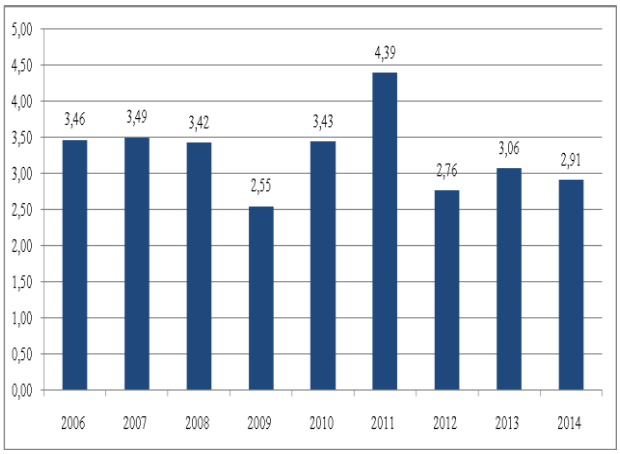

Figure 2. Average producer prices of Raspberry by years (2006-2014)

Strawberry

Strawberry, whose native land is expressed as South America (Chili), is a species which is appropriate for forced growing, has good export and domestic sales possibilities, is herbaceous but a perennial fruit type and also has an increasing spreading in the world with each passing day thanks to numerous rehabilitation studies. USA, Turkey, Canada, Europe, South and East African countries, New Zealand, Australia, and Japan are the countries where strawberry is grown mostly (Anonymus, 2015b). Strawberry cultivation, which started in 1300s A.D. in Europe, contemporarily begun in 1970s in Turkey (Gunduz and Ozdemir, 2008; Ertürk and Geçer, 2012). Turkey plays an important role for strawberry production in the world and takes the 2nd place after USA (Anonymus, 2015b).

A large portion of its production is supplied from Mediterranean, Marmara, and Aegean regions. Mersin is the leading province with the most strawberry production and meets 47% of the total production. Other strawberry producing provinces are Antalya (11%), Aydın (11%), Bursa (9%), and Manisa (6%), respectively (Anonymus, 2015a). Table 2 illustrates strawberry production areas, production amount, and yield levels between 2004 and 2014. Strawberry production areas, which were 97.5 thousand decares in 2004, are generally observed in an increasing trend along with decreases in very small rates by years. Strawberry production area reached 134.2 thousands of da with an increase of 37.68% in 2014 compared to 2004.

Table 2. Strawberry production area, production amount, and yield (Anonymus, 2015a)

Year	Area (decare)	Production(ton)	Mean yield (kg/da)
2004	97.500	155.000	1.590
2005	100.000	200.000	2.000
2006	99.851	211.127	2.114
2007	109.545	250.916	2.291
2008	112.785	261.078	2.315
2009	121.500	291.996	2.403
2010	116.792	299.940	2.568
2011	119.670	302.416	2.527
2012	127.928	351.834	2.750

2013	135.494	372.498	2.749
2014	134.234	376.070	2.802

Production of strawberry, which was 155.000 tonnes in 2004, showed a continuous increase with the influence of increase in the yield in addition to the increase in production areas and reached 376 thousands of tonnes in 2014 with an increase of 142.6% compared to 2004. Owing to use of new technologies and types with high yield in the production, mean yield, which was 1.590 Kg/da in 2004, increased to 2.802 kg/da in 2014 with an increase of 76.23% compared to 2004 (Table 2).

Figure 3 illustrate Agricultural Sector Producer Price Index and corrected strawberry producer prices by months. According to this data, it was observed that producer prices were formed in the period between April and September when strawberry was presented into the market. Accordingly, it was observed that prices were higher in April when the product was relatively lower, lower in June when the product was abundant, and the prices increased again in August by harvesting completely the product. When Figure 4 illustrating 2003-based Agricultural Sector Producer Price Index and annual average take-home pays of the strawberry farmer was examined, it was observed that strawberry prices in this period of ten years were the highest in 2007, the lowest in 2010, and showed increase or decrease depending on export.

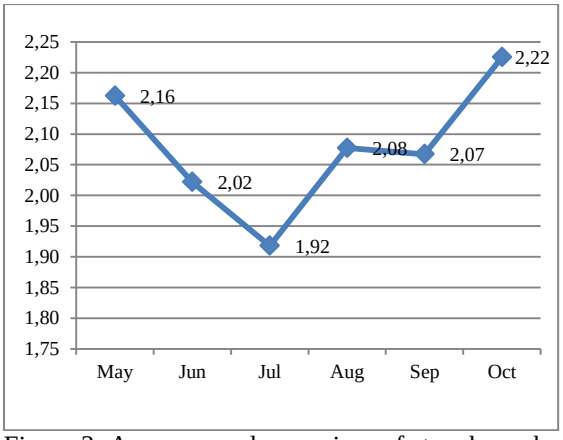

Figure 3. Average producer prices of strawberry by months (2006-2011)

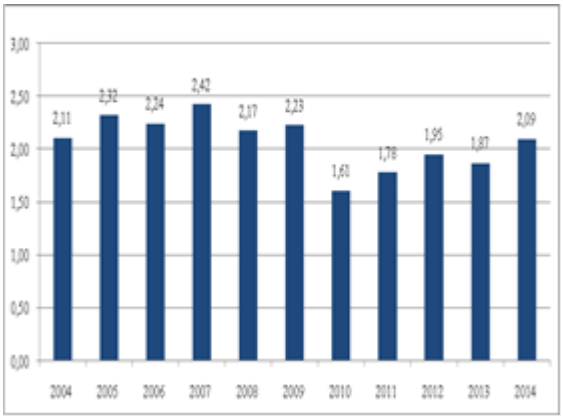

Figure 4. Average producer prices of strawberry by years (TL/Kg) (2004-2014)

In accordance with data of Turkish Statistical Institute, it was observed that 22.360 tonnes of strawberry was exported in 2004-2005 market year, this export increased to 33.000 tonnes as from 2006-2007 market year, reached the highest export level with 37.6 thousands of tonnes in 2009-2010 market year, and 30.3 thousands of tonnes of strawberry was exported in 2013-2014 market year (Table 3).

Table 3. Import, export, and consumption per capita by market years (Anonymus, 2015a)

Marketing year	Imports (Tonne)	Imports-EU 27-28 (Tonne)	Exports (Tonne)	Exports-EU 27-28 (Tonne)	Human consumption per capita (Kg)
2013/'14	492	84	30 279	12 791	4.00
2012/'13	1 401	130	31 926	14 687	3.80
2011/'12	433	48	34 131	16 383	3.20
2010/'11	92	59	34.925	20.477	3.20
2009/'10	167	164	37.627	22.561	3.12
2008/'09	347	148	30.380	18.512	2.88
2007/'08	183	78	35.508	20.212	2.71
2006/'07	161	...	33.011	...	...
2005/'06	303	...	22.158	...	...
2004/'05	-	...	22.360	...	1.67

The exported strawberry changed year by year in small rates by 2007-2008, 56-60% were exported to 27 EU countries, this rate was in tendency to decline as from 2011-2012 market

year, and decreased to 42.2% by 2013-2014 market year. According to data of Turkish Statistical Institute, strawberry consumption was 1.67 kg per capita in 2004-2005 market year in Turkey, increased year by year and reached to 4 kg in 2013-2014 market year (Table 3).

Mulberry

Mulberry grown generally in temperate and subtropical regions of north hemisphere is widely grown in Turkey. White mulberry (97%), black mulberry or red or purple mulberry are common species of mulberry in Turkey. China, India, Turkey, Russia and Middle East countries are known as native land of mulberry (Islam et al., 2006). At the present time, mulberry has an important market potential due to nutritious characteristics of its processed products in addition to consuming it freshly. Tens of products such as molasses, jam, dried fruit roll-up, mulberry paste, dried mulberry, fruit ice cream, fruit leather, vinegar, fruit juice concentrate, spirit are made of its fruit. Especially juice of black mulberry has become a popular drink and a demanded fruit in recent years. In Turkey, mulberry fruits are traditionally used for making molasses and for dry food production by drying. In addition, black mulberry and red mulberry species are used traditionally and at the least commercially for jam industry (Akbulut et al., 2006).

According to 2004 data of Turkish Statistical Institute for areas of collective orchards produced commercially in the direction of industrial demands of mulberry; while 50.000 tonnes of mulberry yielded from 2.130 fruit bearing trees in the area of 11.700 da, areas of collective orchards raised to 20.7 thousand decares by increasing at the rate of 77.55% and the number of fruit bearing trees raised to 2.383 thousand with an increase of 11.90%. The amount of production for the same period reached to 62.879 tonnes in 2014 with an increase of 25.76%. While the lowest production amount within this period of 10 years was observed in 2010, the highest production was observed in 2011(Table 4). While the mean yield per tree was 23 kg in 2004, it raised to 26 kg with an increase of 13.04% in 2014 (Table 4).

Table 4 – Production area, production amount, and yield of mulberry (Anonymus, 2015a)

Year	Area of collective orchards (decare)	Production(tons)	Mean yield per tree (kg)	Number of trees in fruit bearing age	Number of fruitless trees	Total number of trees
2004	11.700	50.000	23	2.130.000	365.000	2.495.000
2005	12.000	55.000	26	2.120.000	366.000	2.486.000
2006	12.797	51.558	25	2.029.207	353.078	2.382.285
2007	13.127	61.665	29	2.094.715	560.426	2.655.141
2008	17.628	65.140	28	2.300.689	539.122	2.839.811
2009	17.029	67.986	28	2.392.609	537.264	2.929.873
2010	18.662	75.096	30	2.479.192	507.465	2.986.657
2011	18.864	76.643	31	2.453.440	359.740	2.813.180
2012	20.500	74.170	30	2.446.907	379.146	2.826.053
2013	21.143	74.600	31	2.422.729	380.047	2.802.776
2014	20.773	62.879	26	2.383.522	379.894	2.763.416

Figure 5 illustrate 2003-based Agricultural Sector Producer Price Index and corrected annual average take-home pays of mulberry producers by months. According to this data, it was observed producer prices formed in the period between May and October when product came on the market. Accordingly, it was observed that the prices were higher in May in which the product was relatively lower, lower in June when the product was abundant, and increased again in July and August as a result of harvesting completely the product, and decreased as from September.

As the Figure 6 illustrating 2003-based Agricultural Sector Producer Price Index and corrected annual average take-home pays of mulberry farmers was examined, it was observed that mulberry prices in this period of ten years were the lowest in 2004 and the highest in 2014.

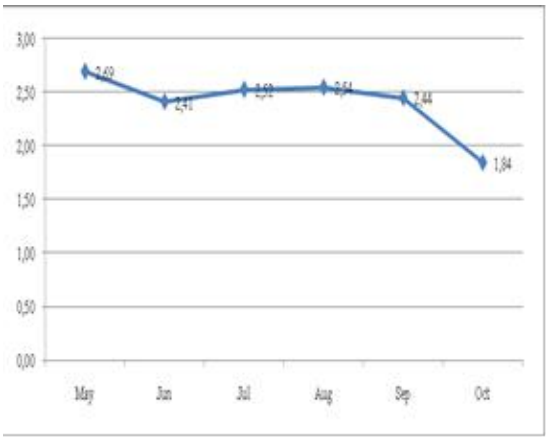

Figure 5. Average producer prices of Mulberry by months (TL/Kg) (2004-2014)

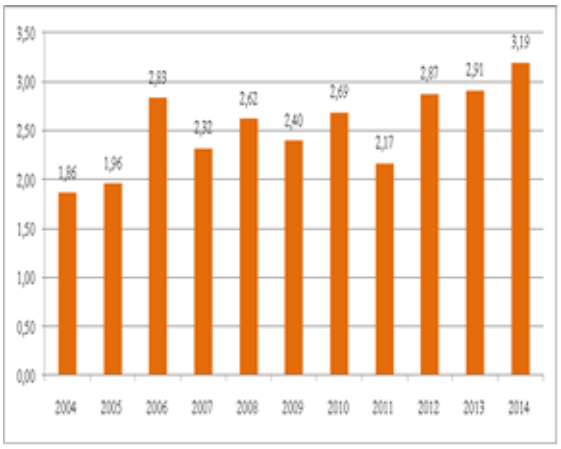

Figure 6. Average producer prices of mulberry by years (TL/Kg) (2004-2014)

In accordance with data of Turkish Statistical Institute stated in Table 5, it was observed that while 258 tonnes of mulberry was exported in 2004-2005 market year, it was 3.751 tonnes in 2013-2014 market year with an increase of 14.54 % compared to 2003-2004 market year. The exported mulberry changed year after year by 2007-2008, and 47-64 % of it was exported 27 - EU countries. Import of mulberry is also performed in order to supply the black mulberry demand of food and drink industry in particular. According to data of Turkish Statistical Institute, mulberry consumption, which was 0.60 kg per capita in 2004-2005 market year, reached 0.77 kg with an increase of 29.35% in 2013-2014 market year in Turkey.

Table 5. Mulberry import, export, and consumption per capita by market years (Anonymus, 2015a)

Market year	Imports (Tonne)	Imports-EU 27-28 (Tonne)	Exports (Tonne)	Exports-EU 27-28 (Tonne)	Human consumption per capita (Kg)
2013/'14	630	79	3.751	2.067	0.77
2012/'13	886	117	1.924	1.238	0.82
2011/'12	2.088	41	1.095	517	0.88
2010/'11	1.232	30	753	473	0.87
2009/'10	519	11	578	293	0.79
2008/'09	685	156	426	242	0.77
2007/'08	469	28	828	490	0.73
2006/'07	1	...	436	...	...
2005/'06	84	...	336	...	...
2004/'05	-	...	258	...	0.60

Cranberry

Cranberry, whose native lands are Anatolia, Caucasia, and Europe, is found generally in wild types as single or groups in coastal and higher parts, mountain sides, and stream beds in Black Sea, Marmara, Aegean, and Mediterranean Regions. Cultivation of graft cranberry is performed in limited amounts in Malatya, Bursa, Yalova, Karabük, and İstanbul provinces (Yalçınkaya et al., 2001). Cultivation of this fruit species, most of which is grown naturally in Turkey, has not been attached importance by the present day (Guleryuz and Pirlak, 1996).

Cranberry is an important source in terms of human health and nutrition. Cranberry fruits are quite much rich in terms of content of vitamin C. Tea and coffee are made of its seeds and leaves. Cranberry is not consumed as fresh fruit much because of its sour and harsh taste and its fruits are utilized for producing gel, compote, jam, marmalade, syrup, alcoholic drink and fruit juice (Kalyoncu and Ecevit, 2005). Essence and drug against dysentery are produced from its fresh peels. It is utilized in numerous forms as dried and table fruit in food industry, as flavouring and aroma giving substance for food and by making compote. Fruits can be conserved for a very long time with the addition of sugar (Ertürk and Geçer, 2012).

According to the data of Turkish Statistical Institute; regarding cultivation area of cranberry commercially produced to supply demands of food industry, 12.000 tonnes of cranberry was

obtained from 1 million trees in fruit bearing age planted in the area of 2.300 da in 2004; whereas, a decreasing trend started by 2006 and 10.982 tonnes of product was obtained from 787.7 thousands of fruit bearing trees planted in 1.522 da area in 2014. Within this period of ten years, the decrease was -34,30% in collective orchard areas, -21,23% in number of trees in fruit bearing age was and -8,48 % in the amount of production compared to 2004. On the contrary of this decrease, there was an increase of 16.67% observed in mean yield per tree; while the mean yield per tree was 12 kg in 2004, 15-16 kg levels were reached in some years and it was 14 kg in 2014 (Table 6).

Table 6. Production area, production amount, and yields of cranberry (Anonymus, 2015a)

Year	Area of collective orchards (decare)	Production(tonnes)	Mean yield per tree (kg)	Number of trees in fruit bearing age	Number of fruitless trees	Total number of trees
2004	2.300	12.000	12	1.000.000	230.000	1.230.000
2005	2.480	11.500	12	981.000	223.000	1.204.000
2006	2.206	9.303	11	878.834	211.773	1.090.607
2007	1.754	9.722	10	947.077	208.088	1.155.165
2008	1.740	11.010	12	942.281	185.967	1.128.248
2009	1.764	14.472	16	889.944	189.085	1.079.029
2010	1.535	12.517	15	820.927	160.130	981.057
2011	1.549	12.427	15	824.118	153.591	977.709
2012	1.873	12.368	15	828.269	150.807	979.076
2013	1.675	11.838	15	810.769	146.747	957.516
2014	1.511	10.982	14	787.709	80.422	868.131

Figure 7 illustrate 2003-based Agricultural Sector Producer Price Index and corrected annual average take-home pays of cranberry farmer. According to these data, it was observed that producer prices formed in the period between April and November when product was in the market. According to means of ten years, it was observed that prices started to increase by the demand of industry beginning from May when the product was abundant and continued to increase in June, this increase was balanced with a relative decrease by July, prices increased again along with the decrease of product amount by October, and reached the highest level in November. When 2003-based Agricultural Sector Producer Price Index and corrected annual average take-home pays of the cranberry farmer (Figure 8) were examined, it was observed that the lowest level of price in period of ten years was 1.72 TL/Kg, as from 2004 producer prices tended to increase along with the changes year by year, reached to the highest level twice with 3.15 TL/kg in 2011 and 2012, it was 2.23 TL/Kg in the following year 2013 and 2.54 TL/Kg in 2014.

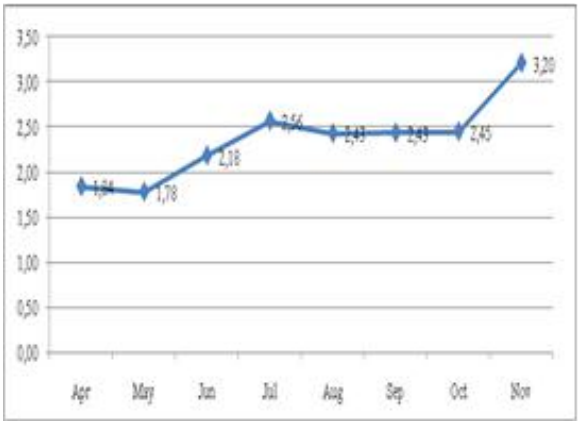

Figure 7. Average producer prices of cranberry by months (2004-2011)

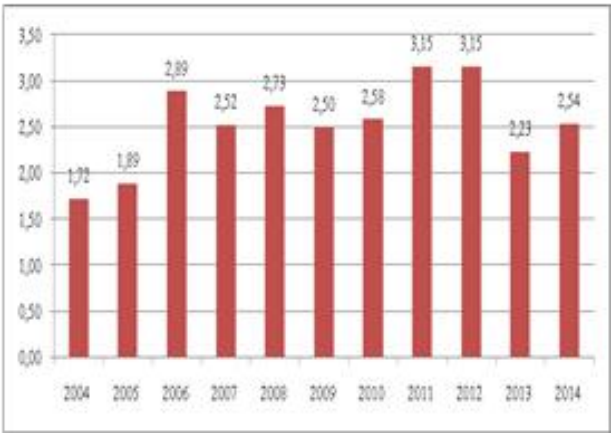

Figure 8. Average producer prices of cranberry by years (2004-2014)

Marketing channels of berry fruits

The process in which property of products is passed into other hands and which includes the steps between producer and consumer is called as marketing channel. Structural development of the sector affects the marketing system, as well. Thus, small-sized enterprises with limited marketing opportunities are generally dominant in agriculture. This situation causes enterprises to carry out insufficiently or never numerous marketing functions such as price formation, standardisation, storage, packaging (Albayrak, 2013). In case that large yield loss and high expenses are observed because of problems resulted from physical processes such as classification, packaging, storage performed for marketing, distribution irregularities and overflow hand-over until agricultural products reach consumer market (Anonymus, 2011). In agricultural product markets; producers, middlemen, brokers, merchants, broker merchants, forwarder merchants, private firms, public agents, commodity exchanges, wholesale food markets, wholesalers, retailers, cooperatives, and producer unions carry on business (Anonymus, 2011; Albayrak, 2013). Major actors playing a role in marketing channels of berry fruits and relationships between them are shown in Figure 9. Sale of products growth by berry fruit producers using organic/traditional methods is performed by themselves or their family members in producer markets, district markets, roadsides through making sale. Another method of sale for the products they grown as with or without a contract is sales they make with local brokers, merchants, farmer associations /cooperatives, manufacturers, and exporters (Figure 9). Local wholesale food market brokers sell the product they buy from producer or producer associations/cooperatives to local retailers, local merchants or directly to brokers in marketplaces of consumption area (Figure 9). Merchants carrying on a business in production area commercialise the products they buy by supplying from producers, producer association /cooperatives, brokers operating in market places of production area to brokers of consumption area, to retailers in production or consumption area (Figure 9).

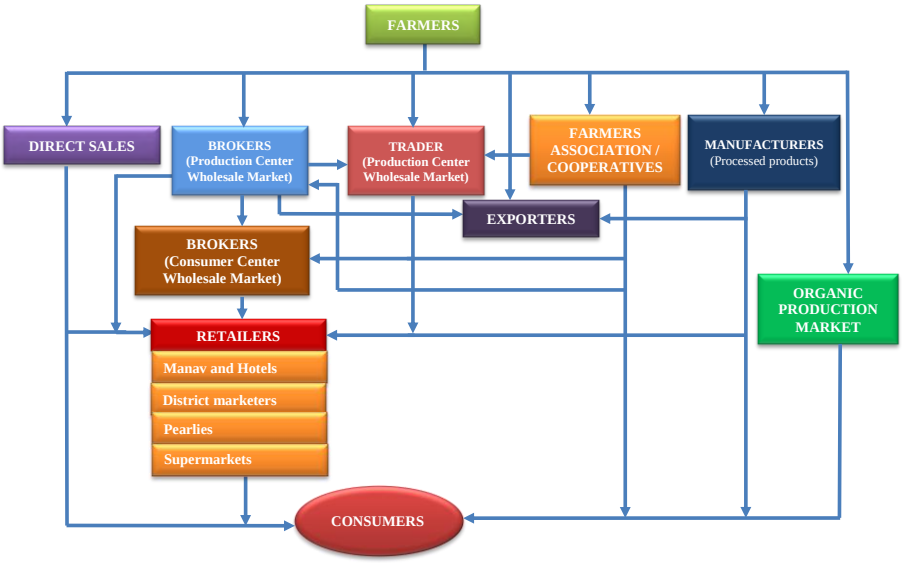

Figure 9. Marketing channels of berry fruits

Exporters supply the raw products they procure from producers and marketplace brokers of production area and the processed products they procure from manufacturers to international market (Figure 9). Producer associations/cooperatives market the product they grow to producers, either directly to consumers or brokers and merchants acting in marketplaces of production area or to retailers (Figure 9). Manufacturers either export products by themselves or transfer them to exporters, after they transform them they supply via contracted production, to finished or semi-finished products by processing. They also make sales via wholesalers or

directly via retailers. Products marketed in market of organic products are directly exported in general or reached to consumers via retailers (Figure 9).

Conclusion and recommendations

When the data of Turkish Statistical Institute is evaluated, it is important to promote increase production of raspberry, strawberry, mulberry, and cranberry species from berry fruits and to establish surrounded gardens in terms of health and welfare of the community. To increase the consumption of these fruits, each of which is virtually a healing capsule, is significant in terms of economy not only by considering health aspects but also because of the added value they create/will create. From production of seedling/sapling to establishment of garden, from establishment of garden to harvesting, labour is heavy, that is, numerous economic activities creating employment are required. It is necessary to pay special attention for the production of these products since they are raw material of many products produced either traditionally or industrially, they are the most interested products in both domestic market and foreign markets, they are the products to have high prices because of high demand. Promoting the production and consumption of these products will considerably increase incomes of small and medium sized enterprises, accordingly be able to embank to migration phenomenon by providing social welfare and peace, enable product diversification to be demanded by consumers, and provide opportunity to fruit processing industry to meet raw material demands.

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Review paper
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LIVESTOCK SUPPORT POLICIES IN TURKEY SINCE 2000

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Abstract

Livestock is essential for both human nutrition and agricultural industries. Hence, governments often apply some support policies in order to develop profitable livestock husbandry and better life. Although the governments applied some statist support policies until 1980, some public support policies have started to be applied after this year. A Supporting Animal Decree numbered as 2000/467 was applied under titles of artificial insemination, calf, equipment, stud heifer, beekeeping and honey, meat and milk, forage, and fisheries in 2000. After this decree, another decree numbered as 2005/8053 was applied for A five-year period in 2005. This decree was cancelled in the following year and it was decided that supports should be decided every year. In this content, "supporting farm animals for each head" policy was preferred and also support of artificial insemination was cancelled. In 2005 a Strategy of Agricultural Policies was applied for agricultural policies between 2006 and 2010. The titles of certified forage seed production, clarifying artificial insemination regions, equipment support for milking hygiene, vaccine, pedigree and conservation native animal genetic resources were determined in this strategy document. Decrees of "Supporting Milk Cows in Southeast Anatolia Region" and "Supporting Beef and Combine Cows in East Anatolia Region" were applied in 2011. Livestock supports in whole supports were 3.4%, 4.4%, 9.2%, 22.3%, 24.4%, 28.5%, 31.7%, 30.5%, and 29% in years of 2000, 2002, 2005, 2010, 2011, 2012, 2013, 2014, and 2015 respectively. In this study legislation based on relavant support policies was investigated by using other researches, articles, thesis, and statistics.

Key words: *Livestock, support, subsidy, agricultural policy.*

Introduction

Livestock has a significant place in Turkish agriculture. The share which value of animal production received from total agricultural production value has gradually increased between 2000 and 2014. According to data of Turkish Statistical Institute; the share of animal production in agricultural production was 44.17% in 2000 and reached to 51.50% in 2009. Value of livestock production continued above fifty percent constantly by receiving a share of 51.50% in 2010, 53.57% in 2011, 56.21% in 2012, 51.49% in 2013 and 52.16% according to provisional data of 2014. The share received by livestock production value within total agricultural production value is accepted as an important indicator along with the other criteria used for determination of development levels of countries (Peşmen and Yardımcı, 2008; Saçlı, 2007). While an investment of at least 80 thousand dollars is required in order to provide employment opportunity for an individual in the industry at the present time, a source which is one fifth of this investment is adequate for livestock. As it is compared to industrial area, employment opportunity can be provided for 5 times more people by livestock with the same amount of investment. Rate of agricultural employment among the total employment in Turkey is a high level like approximately 35% (Demir, 2012). Therefore, livestock also has a vital socio-economic task like preventing the population migration from the rural to urban (Akpınar et al., 2012). Of the area of total 184.329.490 decares in Turkey, 72.38% is in the

possession of enterprises performing both herbal production and livestock, 27.38% is in the possession of enterprises making only herbal production, and 23% is in the possession of enterprises making only livestock (Demir, 2012). All these reasons listed here reveal place and the importance of livestock in the economy of the country. In addition, it makes essential to support livestock when considering the unique importance of animal products in nutrition, mental and physical development of humans. This study investigated support policies carried out for livestock between 2000 and 2015 in Turkey.

Livestock support policies

Livestock sector having considerably high added-value, have been tried to be supported via various policies from 1923 to the present time. Subsidy focused on genetic improvement of native herds, control of diseases, and veterinary services until 1950s (Demir and Yavuz, 2010). When compared to vegetative production industry in the past, desired goals for livestock could not be achieved because livestock supports are not continuous, do not have adequate infrastructure for applying supply policies and accordingly problems of livestock could not be eliminated (Yavuz, 1999, Yavuz et al., 2006, Sayin, 2002).

Livestock policies, which were mainly the public until 1980s in Turkey, were thereafter in tendency to liberalisation, and then came to a state of mostly looking after the interests of parties. Stand-by Treaty concluded with IMF within this scope in 1999 has become the beginning of a new age for agriculture of Turkey (Saçlı, 2007). “The Project of Supporting and Improving Livestock” was initiated in order to eliminate the problems. Within this scope, Decree of Supporting Livestock No.2000/467 was entered into force in 2000 in order to develop livestock and increase animal production. The implementation is a five year decree and involved the years between 2000 and 2004. Along with this decree, incentives were brought for artificial insemination, calfs born by artificial insemination, equipments to use for artificial insemination, animals with breeding certificate, and keeping the stud book records in order to bring the genetic breeding more efficient and common. Furthermore, supports such as breeding heifer support, beekeeping and honey support, support of incentive pay for meat and milk, forage crops support, and fisheries were implemented (Anonymous, 2000). Also, Decision of Partnership Council, which is a milestone for livestock in this period and was concluded in 1998, was also put into force. However, any kind of development has not occurred by 2005 regarding importation of livestock and meat promised to EU within the scope of this decision (Saçlı, 2007). Council of ministers no 2005/8503 with the same content, prepared as a result of studies performed instead of the Decree no 2000/467 expired in 2005, was enacted for a 6 year period. But after one year, this decree was cancelled by Council of Ministers’ Decision no 2008/13489. Thus, it was passed on one-year execution in which supports would be determined again every year, quality and content were changed. A support method as “payment per animal” was preferred within this scope and artificial insemination supports were cancelled (Saçlı, 2012).

In 2005, “Agricultural Strategy Certificate” implemented between 2006 and 2010 as a follow-up version of the decision applied in 2000 was brought to agenda through a council of ministers’ decision. In this policy to be applied, it was aimed for livestock to have a share of 12% among the all supports in 2010. Within this scope, applications regarding the production of certified forage crop seeds, determination of the areas of artificial insemination, equipment support concerning milking hygiene, vaccine support considering animal health, animal identification support, and conservation of gene resources were at the top of the agenda. Supports for sheep and goat breeding took place among the supporting items in which almost all were applied for stock farming in following years (Saçlı, 2007).

In 2006, a legal framework was formed for supports via The Law of Agriculture entered into force by being published in Official Gazette dated 25.04.2006 and numbered 26148. The Law of Agriculture aims the determination of policies and making regulations required for

advancement and support of agricultural sector and rural area in accordance with development plans and strategies. According to the Law of Agriculture, support measures are taken with the aim of race breeding, increasing the production of coarse fodder, increasing the efficiency, specialisation of establishments, providing hygiene conditions in establishments, animal health and welfare, encouragement of animal identification system, processing and marketing animal products, and improvement of related controls, follow-up, and standards and supporting fisheries. Council of Ministers was authorized upon the offer of Council to apply supports and determine the amounts of payments on the basis of regions and provinces in livestock supports (Anonymous, 2006).

Livestock was not imported because Ministry of Agriculture and Rural Affairs did not issue the control certificate for livestock and meat import since 26.8.1996 on the account of the fact that "foot and mouth disease existed in Turkey and health of imported animals could not be protected because of the illness in our country" (Kandemir, 2010). Along with the emergence of Bovinge Spongiform Encephalopathy (BSE) in 1996 in international arena, butchery animal and red meat were not imported from 1997 to 2010 in Turkey (Aydın et al., 2010). However, while gradually increase of the population against the decrease in number of animals reduced the meat production per capita, meat prices considerably rose. However, a reel increase in red meat prices firstly appeared for sheep-goat meat as it came to the 2nd half of 2008, which was followed by the cattle meat in the 2nd half of 2009. Ministry of Agriculture and Rural Affairs (TKB) wanted to balance the reel price increase of red meat (Aydın et al., 2010). The task for butchery animal import was assigned to General Directorate of Meat and Fish Authority, in 2010 in the first step to decrease red meat prices having a tendency to a continuous increase from the second half of 2009. Import permit for stock material and carcass meat was given to private sector by deducting custom duties after then because a decrease was not observed in prices (Anonymous, 2011). In this period, import of livestock, meat and breeding animal was started in Turkey and both custom duties and reference prices and import countries and technique specifications of animals to import have been changed for several times from 28 April 2010, when the first tender bid was announced, to 2014 (Akman, 2011). The last change made in custom tariff entered into force by being published on Official Gazette dated 30 October 2012 and numbered 28452. Proportions of custom duty for cattle carcass meat increased from 75% to 100%, for butchery male calf from 15% for other cattle from 0%, for lamp and sheep from 20% to 40%, by Council of Ministers' Decision No 2012/3758 (Anonymous, 2014).

When it came to 2011; livestock supports started in 2000s considering various issues in bovine and ovine breeding were continued by "Council of Ministers' Decision Regarding Agricultural Supports to be performed in 2011" Numbered 2011/1430. With the given decision; support payments were made in the subjects of brood cattle-buffalo, sheep-goat, calf born via artificial insemination, hybrid born via artificial insemination, raw milk, raw milk assessment, sheep-goat breeding project, feeder male cattle, protection of gene resources, angora, silk worm, beekeeping and fisheries, coarse fodder production, returned animal disease, disease free establishment, struggling with animal disease, programmed vaccine practices. In addition, several political tools were enabled such as "Decision Regarding Supports of Dairy Cattle Breeding Investments in Provinces within the Scope of Action Plan of Southeastern Anatolia Project " and "Decision Regarding Supporting the Investments of Breeding Cattle Establishment to Built with Beef and Combined Cattle Breeds in Provinces within the Scope of Eastern Anatolia Project " (Saçlı, 2012).

Payments regarding certified breeding heifer support, incentives of artificial insemination, incentives of artificial insemination calf, incentives of artificial insemination equipments, silk worm cocoon support, angora support, hatched chick support, avian influenza disposal compensation, beekeeping and honey support, meat incentive pay, milk incentive pay,

disease-free establishment support, forage crops, machinery support, seed support for certified forage crops, milking hygiene and milk quality, animal gene resources, animal identification system, struggling with disease (vaccine), food safety, ovine breeding association support, fisheries support, and bovine support has been provided to breeders since 2000 within the scope of livestock supports (Demir and Yavuz, 2010).

Support payments of livestock

Table 1 illustrates that agricultural supports between 2000 and 2015 reached to 4 billion dollars from 1.48 billon dollars by increasing 4 times in 2000. At the same period, livestock supports increased to 1.16 billon dollars from 19 million dollars by increasing 60 times. When it came to share of livestock supports among the total supports by years, the share was 1.29% in 2000 and reached to 21.8% in 2015 with an approximately increase of 22.5 times. While rate of increase of livestock supports compared to the previous year was examined, we can assert that it increased with higher rates compared to total agricultural supports (Table 1).

Table 1- Total agricultural support payments and livestock supports * (Anonymous, 2015a)

Years	Total agricultural supports (Million TL)**	Total agricultural supports (Million $)	Livestock supports (Million TL)**	Livestock supports (Million $)	Share of Livestock supports in Total agricultural supports (%)	Increase rate of agricultural supports compared to previous year (%)	Increase rate of livestock supports compared to previous year (%)
2000	**927**	1.486	**12**	19	1.29		
2001	**1.032**	842	**49**	40	4.75	0.10	0.76
2002	**2.276**	1.511	**75**	50	3.30	0.55	0.35
2003	**3.105**	2.080	**126**	84	4.06	0.27	0.40
2004	**3.084**	2.169	**209**	147	6.78	-0.01	0.40
2005	**3.708**	2.765	**345**	257	9.30	0.17	0.39
2006	**4.793**	3.349	**661**	462	13.79	0.23	0.48
2007	**5.643**	4.334	**741**	569	13.13	0.15	0.11
2008	**5.864**	4.535	**1.095**	847	18.67	0.04	0.32
2009	**4.749**	3.070	**908**	587	19.12	-0.23	-0.21
2010	**5.947**	3.965	**1.158**	772	19.47	0.20	0.22
2011	**7.085**	4.243	**1.728**	1.035	24.39	0.16	0.33
2012	**7.673**	4.279	**2.216**	1.236	28.88	0.08	0.22
2013	**8.774**	4.615	**2.756**	1.450	31.41	0.13	0.20
2014	**9.599**	3.303	**2.887**	993	30.08	0.09	0.05
2015	**10.141**	4.005	**2.953**	1.166	29.12	0.05	0.02

* Complied by authors. ** With current prices

Figure 1 illustrates payments of agricultural and livestock supports. It was observed that supports were in increasing trend despite decreases in 2009 and 2014.

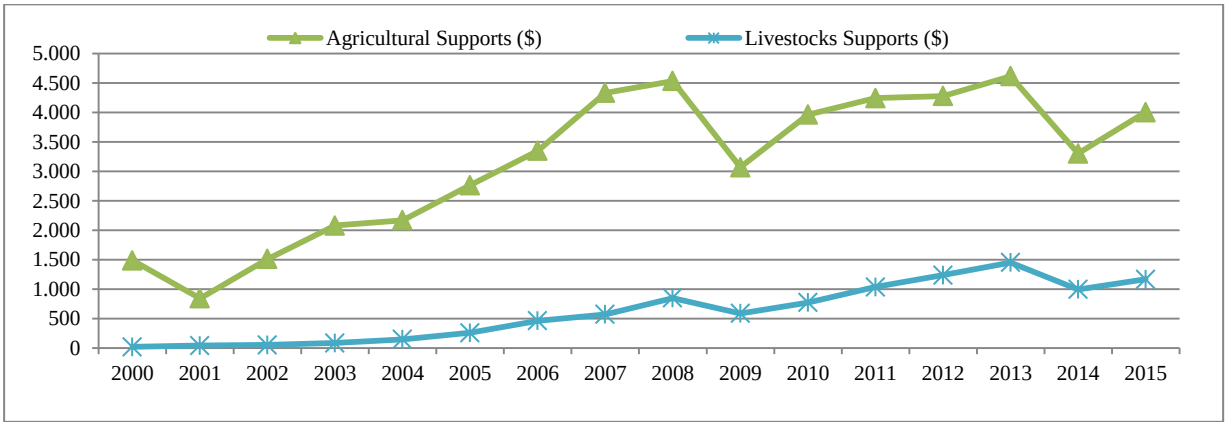

Figure 1 – Total agricultural supports and livestock supports (Anonymous, 2015)

Figure 2 illustrates the share of livestock supports within total agricultural supports. Whilst it was observed that share of livestock supports within total agricultural supports was increasing

gradually, reached to the highest level in 2013 with an increase of 31.41%, went back to 30.08% in 2014, and it is estimated to decrease to 29.12% in 2015 (Figure 2).

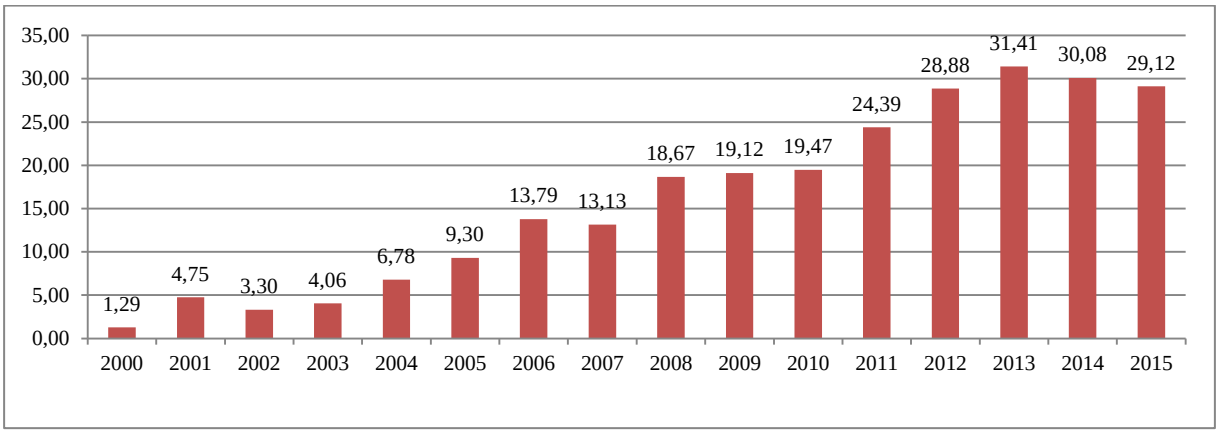

Figure 2 – Share of livestock supports within agricultural supports (%),(Anonymous, 2015a)

Annual increase rates for total agricultural supports and livestock supports from these supports were compared at Figure 3. Annual increase rate for livestock supports increased in a higher level compared to total agricultural supports. But, it can be asserted that I has followed a parallel trend since 2009 when a negative increase was observed compared the previous year. Although share of livestock supports increased to 30% after 2010, rate of increase has shown a gradually decreasing structure as from 2011.

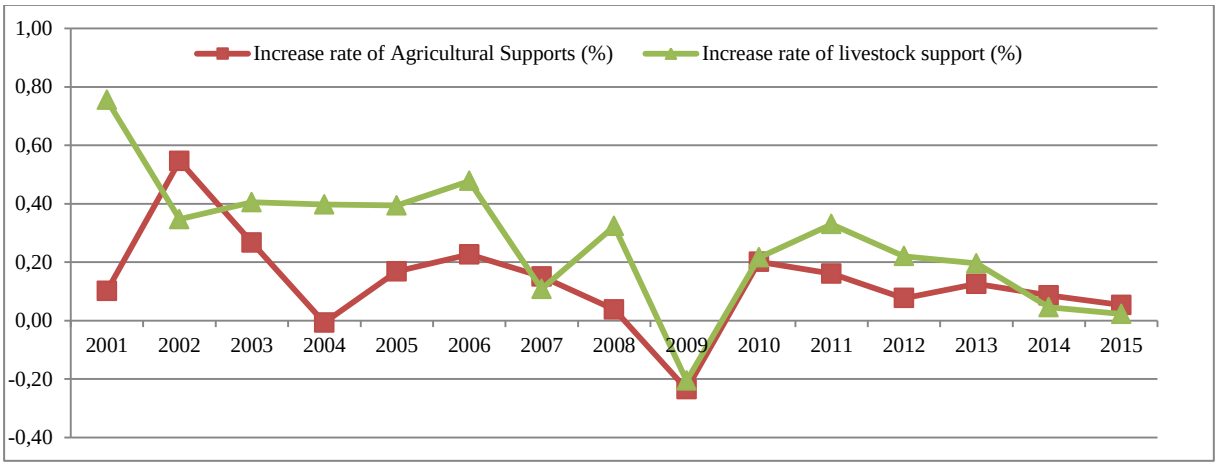

Figure 3 – increase rate of agricultural and livestock supports compared to the previous year

Changes in presence of main livestock and major animal products in turkey between 2000 and 2014

This section examined the changes occurred in number of livestock in return for the increase of share of livestock supports within total supports. Giving permission for livestock import in 2010 and providing agricultural credit with interest of 0% for animal production activities via Ziraat Bank and Agricultural Credit Cooperatives (TCC) as from 2011 have caused increase of animal number and animal prdocution. (Hatunoğlu and Eldeniz, 2012).

It was observed that while the number of cattle was 10.7 million heads in 2000, it reached to 14.1 million heads with an increase of 23.8% compared to 2000 due to the positive effect of increasing supports as from 2010 (Table 2). The number of buffalos, which was, 146.000 in 2000 decreased dramatically because buffalo breeding was underestimated. Even though it showed an increasing trend again after 2010, 2014 was entered with 121.8 thousand heads of buffalo with a decrease of -19.4% compared to the number of buffalo in 2000 (Table 2). The

number of heads of sheep, which was 28.4 millions in 2000 in Turkey, decreased to 25.1 millions in 2002. The number of sheep, which was almost 25 million heads until 2008, reduced to 21.7 million heads in 2009 by showing a decreasing trend. The effect of supports increasing as from 2009 was started to be observed and the number of sheep in Turkey reached to 31.1 million heads with an increase of 8.43% in 2014 compared to 2000. The number of goats, which was 7.2 millon heads in 2000, entered to a decreasing trend ongoing until 2009 and reduced to 5.1 millon heads. With the effect of supports given as from 2009, an increasing trend started again. Thus, the number of goats in 2014 reached to 10.3 million heads with an increase of 30.41% compared to 2000.

Table 2 – The number of major Livestock in Turkey between 2000 and 2014 (Anonymous, 2015b)

	Cattle	Buffalo	Sheep	Goat
2000	10.761.000	146.000	28.492.000	7.201.000
2001	10.548.000	138.000	26.972.000	7.022.000
2002	9.803.498	121.077	25.173.706	6.780.094
2003	9.788.102	113.356	25.431.539	6.771.675
2004	10.069.346	103.900	25.201.155	6.609.937
2005	10.526.440	104.965	25.304.325	6.517.464
2006	10.871.364	100.516	25.616.912	6.643.294
2007	11.036.753	84.705	25.462.293	6.286.358
2008	10.859.942	86.297	23.974.591	5.593.561
2009	10.723.958	87.207	21.749.508	5.128.285
2010	11.369.800	84.726	23.089.691	6.293.233
2011	12.386.337	97.632	25.031.565	7.277.953
2012	13.914.912	107.435	27.425.233	8.357.286
2013	14.415.257	117.591	29.284.247	9.225.548
2014	14.122.847	121.826	31.115.190	10.347.159

Figure 4 illustrates increase rates in number of cattle, buffalo, sheep, and goat between 2000 and 2014 compared to the previous year. When the figure was examined, supports given at Figure 3 changed in parallel to annual increase rate. It can be asserted that the lowest rate of increase was seen as a negative growth in 2007 for buffalo breeding, in 2008 for cattle and goat and in 2009 for sheep. It was observed that livestock supports increasing as from 2009 affected the increase in the number of goats at the most and a decreasing trend started in increase rates after 2012 (Figure 4).

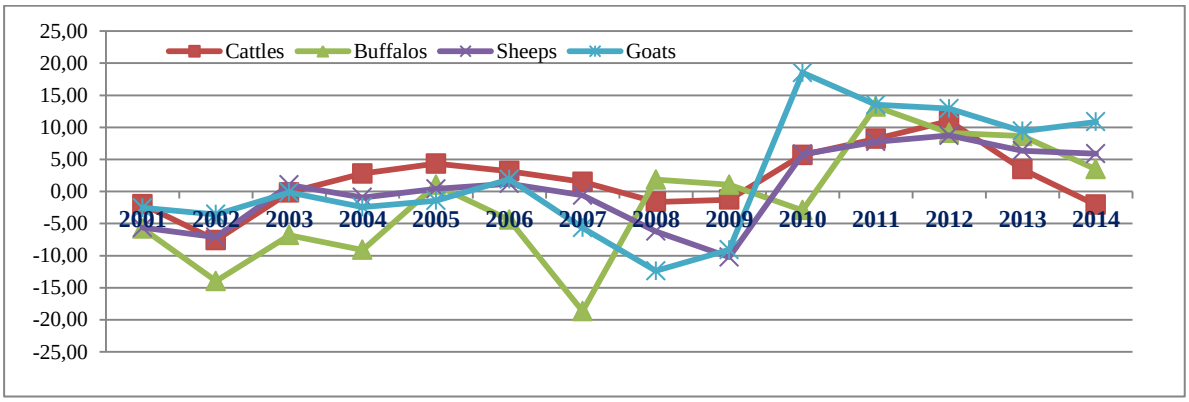

Figure 4 – Increase rate in number of major livestock compared to the previous year

Table 3 illustrates changes of some animal products between 2000 and 2014. It was observed that there was a considerable increase in animal production as a result of policies implemented for decreasing the price increase observed in the market of meat products sector after 2009 (Hatunoğlu and Eldeniz, 2012).

Red meat production, which was 491.5 thousand tons in 2000, increased more than 1 million tonnes with an increase of 49.29% in 2014 compared to 2000 (Table 3). Milk production,

which was 9.79 million tonnes in 2000, increased to 18.49 million tonnes with an increase of 45.5% compared to 2000 in parallel to supports increasing as from 2009 (Table 3). Chicken production, which was 643.45 thousand tonnes in 2000, and reached to 1.89 millon tonnes with an increase of 68.5% in 2000 by replacing red meat with chicken meat upon high red meat prices (Table 3). Chicken egg production which was 13.5 billion in 2000, reached to 17.14 billion in number with an increase of 17.82% in 2014 compared to 2000 (Table 3). When honey production was examined, honey production, which was 61 thousand tonnes in 2000, showed an increasing trend at lower levels for long years and reached to 102 thousand tonnes with an increase of 34.37% in 2014 compared to 2000 (Table 3).

Table 3- Main Animal products in Turkey between 2000 and 2014 (Anonymous, 2015b)

	Red meat (Ton)	Milk (Ton)	Chicken (Tonnes)	Chicken egg (1000)	Honey (Tonnes)
2000	491.499	9.793.962	643.457	13.508.586	61.091
2001	435.778	9.495.550	614.745	10.575.046	60.190
2002	420.595	8.408.568	696.187	11.554.910	74.554
2003	366.962	10.611.011	872.419	12.666.782	69.540
2004	447.154	10.679.406	876.774	11.055.557	73.929
2005	409.423	11.107.897	936.697	12.052.455	82.336
2006	438.530	11.952.099	917.659	11.733.572	83.842
2007	575.622	12.329.789	1.068.454	12.724.959	73.935
2008	482.458	12.243.040	1.087.682	13.190.696	81.364
2009	412.621	12.542.186	1.293.315	13.832.726	82.003
2010	780.718	13.543.674	1.444.059	11.840.396	81.115
2011	776.915	15.056.211	1.613.309	12.954.686	94.245
2012	915.844	17.401.262	1.723.919	14.910.774	89.162
2013	996.125	18.223.712	1.758.363	16.496.751	94.694
2014	1.008.272	18.498.630	1.894.669	17.145.389	102.486

Figure 5 illustrates the increase rates in production amounts of animal products between 2000 and 2014 in comparison with the previous year. It was observed in case that there was a change in parallel to annual increase rate of supports given in Figure 3. We can tell that the of highest production level of animal products decreased in 2010 by reacting to 2009 when share of livestock supports started to increase and as from this year it entered into a decreasing trend (Figure 5).

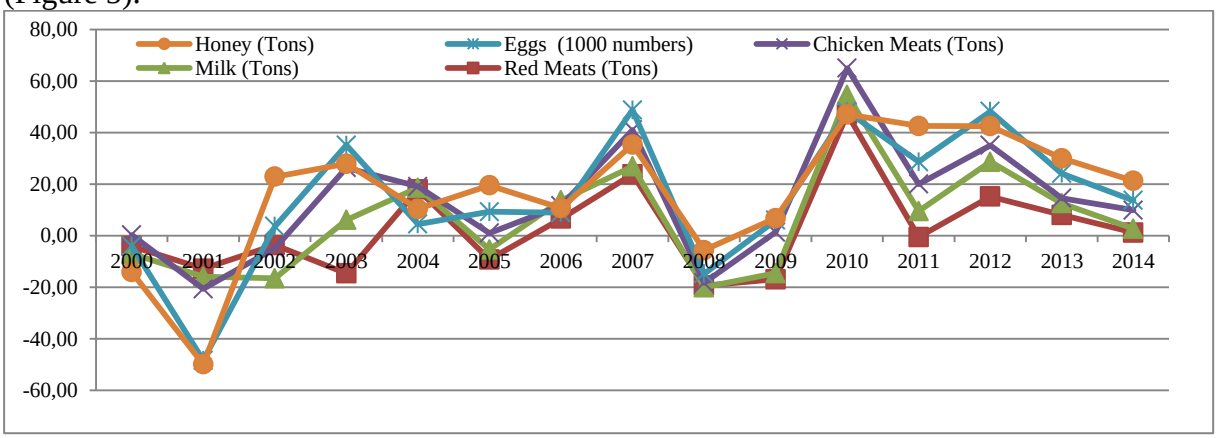

Figure 5 – Increase rate of main animal products compared to the previous year

Conclusion and recommendations

Through livestock supports implemented from 2000 to the present day; measures for environmental precautions have been put into practice along supports regarding meat premium, marketing support, modernisation of livestock enterprises in addition to current support instruments for the purpose of improvement of race breeding, improvement of coarse fodder production, increase of yield, specialisation of establishments, providing hygiene conditions at establishments, animal health and welfare, incentive of animal identification

system, processing and marketing animal products supports and improvement of related controls, follow-up, and standards.

It was aimed to increase the number of livestock establishments specialized in the sub-sector of livestock through new supports. Share of livestock supports in agricultural support budget increased to 30% as a result of growing within the scope of implementation.

According to 2000-2014 data from Turkish Statistical Institute, number of livestock and production of animal products responded to support policies and thus increased and policies were successful even though they were not sufficient enough to meet the demand of increasing population. But, the fact that changes in production are supersensitive to changes in supports indicates structural problems of livestock establishments have continued. Livestock supports need to turn to construction and improvement of permanent end sustainable-sized enterprises.

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FORESTRY AND AGRO-FORESTRY

Original scientific paper
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VERIFICATION OF IMPORTANCE OF THE FORESTRY MANAGEMENT FOR THE RIVER WATER CONSERVATION IN AGRO-FORESTRY WATERSHED

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Abstract

Nitrogen pollution of river water due to agricultural activities was concerned in the Tokachi River basin in the eastern Hokkaido, Japan. Meanwhile, the riparian forests and windbreak forests in agricultural area exists as a buffer zone of nitrogen export and a migratory path and breeding site for wild animals. Here, we examined the relationships between the forestland in the watershed and the nitrate nitrogen (NO_3-N) concentration in the river water. Also, we discussed the importance of forestry management for the implementation of sustainable agriculture and the biodiversity conservation.

The main stream and the tributaries of the Tokachi River basin were monitored in 2007-2011 and the NO_3-N concentrations in the river water were measured. The proportion of forestland and connectivity of forestland (the spatial continuity (*SC*) of forestland) were analyzed by the GIS. NO_3-N concentrations were negatively correlated with the proportions and *SC* of forestland. It was considered that the agricultural land runoff, which contained a lot of nitrogen was diluted by the forestland runoff which had low nitrogen concentration. In particular, the correlation coefficients between the *SC* of forestland and the NO_3-N concentrations were high.

There is a possibility that the NO_3-N concentrations decreased by not only the large forestland in upper stream but also by the windbreak forests or riparian forests, which have high connectivity in the agricultural area. It is important to manage the forestlands properly for suppression the nitrogen export from the agricultural land in the Tokachi River basin.

Keywords: *Forestry management, River water quality, NO_3-N concentration, The Tokachi River basin*

Introduction

Forests serve multiple functions including provision of timber products, land conservation, watershed protection and ecosystem service such as conservation of biodiversity (Erwin, 2000; Pistorius et al., 2012). The Tokachi River basin in eastern Hokkaido, Japan has an important role as a food base of Japan. Forestland accounts for 60% of the watershed area in this basin as well. The native and mountain forests in the upper stream of the Tokachi River are conserved areas. However, the riparian and windbreak forests in the lowland areas have reduced in the last couple of decades because of their conversion to agricultural land and the decrease in wood prices (Tsuji et al., 2005). Forests in the lowland area represent a valuable habitat and serve as a migratory path for the wild animals in an agricultural area (Strasser and Lang, 2015). Riparian forests are expected to remove some nutrient salts from the groundwater and river water (Hubbard et al., 1997).

In addition, the Tokachi River basin has been assessed as one of the basins with the highest nitrogen load from agricultural activities in Hokkaido, Japan (Woli et al., 2004; Yamazaki et al., 2013, 2014). Forest ecosystem functions such as water quality conservation have attracted attention in recent years. Enrich (1996) and Moore and Allen (1999) suggested that

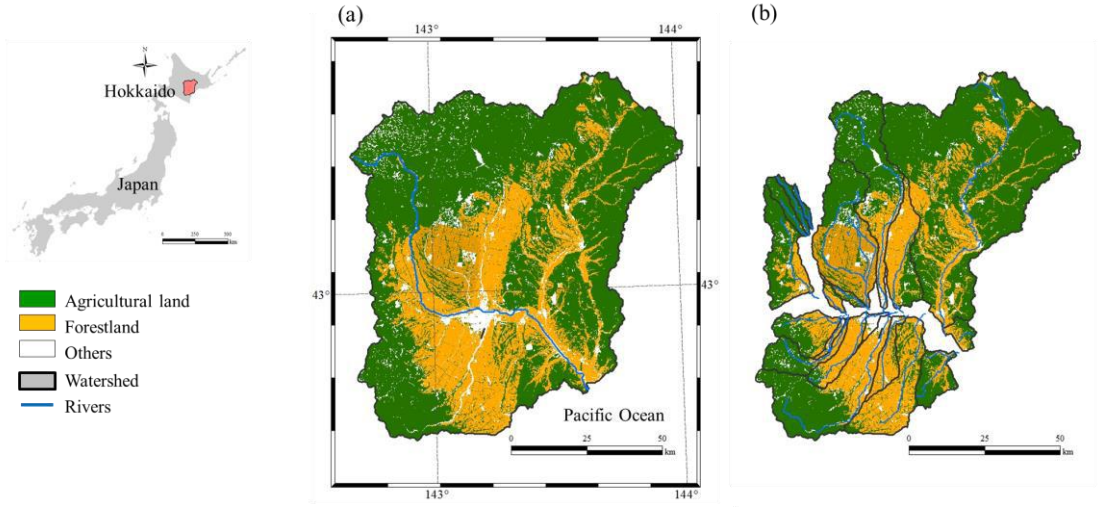

Fig. 1 The Tokachi River basin: (a) Land use andwatershed boundaries of the main stream; (b) Land use and watershed boundaries of the tributaries

biodiversity conservation in forests is strongly related to ecosystem function and stability.
To identify ecosystem functions, it is necessary to evaluate not only the area but also the distribution of forestland. For example, connectivity (agglomeration) of forestland is emphasized in forest ecosystems (Forman, 1995; Schumaker, 1996).
In this study, we evaluated the impact of forestland on nitrate-nitrogen (NO_3-N) concentrations in the river water of the Tokachi River basin. We also focused on the proportions and agglomeration of forestland. We discussed the importance of forestry management for the implementation of sustainable agriculture and ecosystem services.

Material and methods

Study area

An outline of the study area in the Tokachi River basin (142.68–144.02°N, 42.55–43.65°E, 0–2,077 m altitude) in eastern Hokkaido, Japan is shown in Fig. 1. The basin consists of the main stream and approximately 20 tributaries. The main land uses in this river basin are agriculture and forests. Of the agricultural land, 70% is used as cropland and the remaining 30% as pasture. The low-lying areas around the tributaries are used as croplands. The crop cultivation period is from May to November. Chemical fertilizers and compost are used in the agricultural land.
The headstream of the Tokachi River is designated as a nature conservation area, and a virgin habitat is maintained for rare species. Mixed forests of conifers and broad-leaved trees comprise the native forests of this basin. Riparian and windbreak forests have been planted in the low-lying areas of this basin to prevent soil and wind erosion of the agricultural land. *Salix pet-susu*, *S. rorida*, *S. gracilistyla* and *Chosenia arbutifolia* are the main tree species in the riparian forests. In particular, *C. arbutifolia* has a disjunct distribution limited to Hokkaido and Nagano prefectures. The main tree species in the windbreak forests in the Tokachi River basin are *Larix kaempferi*, *Betula platyphylla sukachev* var. *japonica*, *Abies sachalinensis* and *Picea glehnii* (Tsuji et al., 2005). The meteorological environment in the Tokachi River basin is classified as a warm summer continental climate type (Dfb) according to the Köppen–Geiger climate classification (Peel et al., 2007). The annual mean air temperature and precipitation, measured at Obihiro city from 1981 to 2010, were 6.8 °C (minimum −7.5 °C in January; maximum 20.2 °C in August) and 887.8 mm/y, respectively.

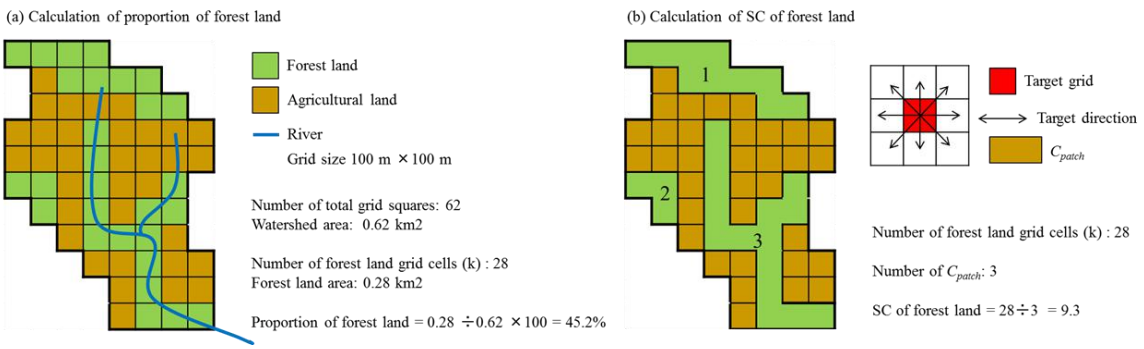

Fig. 2 Calculation methods for (a) proportion and (b) spatial continuity (SC) of agricultural land

Water quality investigation

Sampling points were located on the main stream (points 1–17) and downstream of each tributary (A–T). Thirty-seven sampling points were monitored in June, August or September and October from 2007 to 2011 under base flow conditions. NO_3-N was analysed by the chromotropic acid or cadmium methods.

Land use analysis

The GIS software application ArcGIS Desktop (ver.10, ESRI) was used for land use analysis. ‘River line’, ‘watershed boundary’ and ‘land use raster (100 m grid size)’ data provided by the National Digital Information were used to construct a land use map of the Tokachi River basin. The proportions and the spatial continuity (SC) of agricultural land were used for land use analysis. The proportion of agricultural land is the area of agricultural land in a watershed divided by the watershed area (Fig. 2a). SC was proposed by Tsunekawa et al., 1991. Okazawa et al (2011) investigated the relationship between SC of agricultural land and forestland in the Tokachi River basin and T-N concentration on a trial basis. To calculate the *SC* index, the land use grid squares that were vertically, horizontally and diagonally connected were grouped into a patch (Fig. 2b). *SC* values in the watershed were obtained by Eq. (1).

$$SC = k/C_{\text{patch}} \qquad (1)$$

Where k is the total number of grid squares of agricultural land in the watershed, C_{patch} is the number of patches in the watershed. SC evaluates the area of connected identical land use grid squares in the watershed.

Results and discussion

The relationships between the proportion or SC of forestland in the watershed and the NO_3-N concentrations in the river water are shown in Fig. 3 (a) and (b). NO_3-N concentrations are average values during the study period. The proportion of forestland in the watershed and the NO_3-N concentrations in the river water showed a negative correlation (r = 0.87) (Fig. 3 (a)). The NO_3-N concentrations tended to be low in watersheds with high proportions of forestland. However, NO_3-N concentrations varied from 1.9 to 5.7 mg/L in watersheds with ≤40% proportions of forestland.

NO_3-N concentrations in the river water showed a negative correlation (r = 0.92) with the SC of forestland (Fig. 3(b)). The correlation coefficients of SC of forestland were higher than those of the proportion of forestland. This result suggested that the NO_3-N concentrations tended to be low in the watersheds with high connectivity of forestland even if the proportion of forestland was the same.

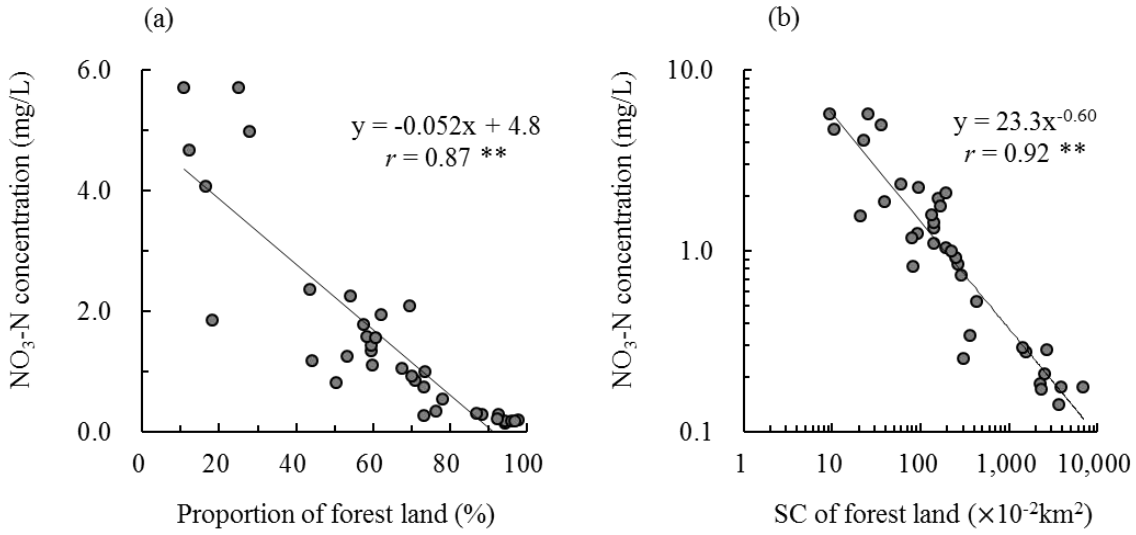

Fig. 3 (a) and (b) Relationship between the Proportion and SC of forestland and the NO_3-N concentrations in the Tokachi River basin. ** shows significance level ($p < 0.01$)

Generally, NO_3-N concentrations in mountain streams were low. Consequently, a dilution effect on the river water NO_3-N concentrations, by mountain streams, was found as a function of forestland in agricultural watershed. The NO_3-N concentrations in upper streams of the Tokachi River with forestland of ≥90% showed very low values (≤0.2 mg/L).

It was inferred that the connectivity of forestland was extended by the riparian and windbreak forests in lowlands of the Tokachi River basin. Riparian forests have abundant soil moisture and relatively nutrient reducing conditions (Robert et al., 2009). Denitrification and plant uptake in the riparian forest are thus thought to remove nitrogen from shallow groundwater (Nakamura et al., 2005). On the other hand, there are few studies on control of nitrogen runoff from agricultural land by windbreak forests. However, we speculate that the biodiversity in the windbreak forests has the potential to consume or remove surplus nitrogen from agricultural land.

Conclusion

There was an effect of decreasing the NO_3-N concentrations in the river water by forestlands in the Tokachi River basin. The results suggested that increasing the connectivity of forestlands, especially the forests in lowland area was effective to remove the nitrogen loads from agricultural activities and also to conserve the biodiversity.

From these results, we should preserve not only mountain and native forests but also forests in agricultural land such as riparian and windbreak forests to bring out the multi functions of forests in agricultural watershed. It is necessary to investigate the ecosystem function and effects on environments of forestlands in detail to preserve forestlands in agricultural watershed with sustainable agricultural productivity.

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CONDITIONS FOR THE INITIATION OF MOTION AND TRANSPORT OF SEDIMENT IN TORRENTIAL WATERCOURSES

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Abstract

Eroded material in torrential watercourses moves in waves of maximum discharge. The paper deals with studies related to the impact of geological type of stream bed in a drainage basin on the rate of rock erodibility, fragmentation and hydraulic transport through the catchment hydrographic network. The study was performed in the area of the Teaching Base of the Faculty of Forestry from Belgrade, on the river Gvozdacka Reka on Mt. Goc, in the Zapadna Morava River basin. A reservoir was created at the profile of the Gvozdacka Reka tributary. Water was released from the storage to simulate floodwave discharge in the watercourse. The experiments were repeated by successive releases of water from the storage and suspended sediment concentrations were calculated. Maximum discharges were measured at the overflow, at the beginning of the experimental stream reach. The characteristics of eroded material along the riverbed were defined, based on the geomechanical and granulometrical analysis of sediment from the profile and banks of the riverbed at the experimental stream reach. Using standard methods related to the conditions for the initiation of motion of bedload and suspended load, critical tractive forces for each sediment fraction from the structure of the stream profile were calculated.

Key words: *sediment particle-size composition, bedload, tractive forces.*

Introduction

River sediment is composed of particles of different sizes, from dust to bpulders. Depending on boundary shear stress, energy gradient, flow velocity and particle-size, river sediment moves in the form of:

Bedload, usually 5-15%, though it can vary from 100% to less than 0.1% (Galy and France-Lanord, 2001; Babinski, 2005; Kazemi et al., 2012);

Suspended sediment, 85-95%, (Jovanovic, 2008).

The characteristics of eroded material (fraction sizes and roundness) in drainage basins of hill and mountain streams primarily depend on bedrock type and rock resistance to weathering. Rounding of sediment particles depends on the rock composition and hardness and the distance travelled from the source the depositional area (Letic, 1981). The movement of eroded material is a continuous process with varying intensity and it is directly related to flow and water level variations in the stream cross section. (Bakker, 2005) Sediment movement is a very complex natural process and its study requires the application of almost all modern scientific methods in the fields of fluid mechanics, water and sediment turbulent movement, stochastic analysis of peak flows and sediment transport through the drainage pattern (Globevnik, et al, 1998).

Although the study of bedload entrainment and movement has been the subject of scientific interest for a long time, the adequate results in the professional practice are still insufficient for the solution of current engineering issues (Jovanovic, 2002; Martha et al, 2005).

The transport of suspended sediment is commonly monitored at the selected sites by standard procedures. While bed material moves beyond a given shera stress threshold during flood waves, suspended sediment, and especially its wash load component, is transported also during the lower flows of the flood hydrograph (Lamakin, 1950).
Bedload moves sporadically and randomly on the river bed in response to local flow conditions which can be investigated at selected sites (Kondolf et al, 2002).
There is not a strictly defined threshold between bedload and suspended sediment grain size. The same particle sizes that, in the given hydrological-hydraulic conditions, move as bedload can become suspended sediment, depending on hydraulic forces, increased discharge and tractive forces (Karasaev, 1975; Ortigosa and Ruiz Flano, 1995).
Sediment management relies on the quantitative data measured in the field as sediment transport equations predict transport rates which are commonly even a few orders of magnitude far from the actual ones. The necessary data should be measured in natural streams as input data for contour conditions (Keesstra et al, 2003).
To give a contribution to the solution of particle entrainment conditions in natural streams, this study was carried out on the the main stem of the Gvozdacka Reka ("*reka*" means river in Serbian language) (Fig. 3 and 5) and its tributaries in the experimental reach about 250m long (Kantoush and Sumi, 2010), in the area of the Faculty of Forestry Teaching Base of Belgrade on Mt. Goc (Serbia).
The Gvozdacka Reka tributaries, Bela Reka and Prerovska Reka, deliver great quantities of eroded material from the catchment to the zone of the Teaching Base on Mt. Goc and silt up the check dams constructed in the streams (Fig. 2). The vegetation of the study drainage basins is characterised by good canopy and coverage (>95%), so there is no visible evidence of runoff erosion, except for gravel roads and the input of sediment of organic origin. However, fluvial erosion processes are present, witnessed by bank scouring and bank collapse (Rogers & Schumm, 1991). The organo-mineral sediment moves permanently and periodically through the stream network and it is transported as suspended load and bedload, respectively (Fig. 4) (Ortigosa and Ruiz Flano, 1995).
The entrained sediment is deposited upstream of natural obstacles or the check dams. The essential function of the dams in the catchment is to control the bedload movement and also to protect the storage intended for trout fishpools, which belongs to the Teaching Base, and to conserve water quality for downstream users. (Suresh Kumar et al, 2012).

Material and methods

The study was carried out on the Gvozdacka Reka, which is located in the Zapadna Morava River basin (Fig. 1).
The bedrock underlaing the Gvozdacka Reka catchment consists of dacites and andesites, mainly from the Tertiary, Mesozoic porphyries and porphyrites, with the following distribution:
Serpentines, serpentinites 11.02%;
Harzburgites, granodiorites, 35.00%;
Sandstones, dacites and andesites, alevrolites, marbles 8.75%;
Schists, mixed serpentinites and metabasalts, 45.23%.

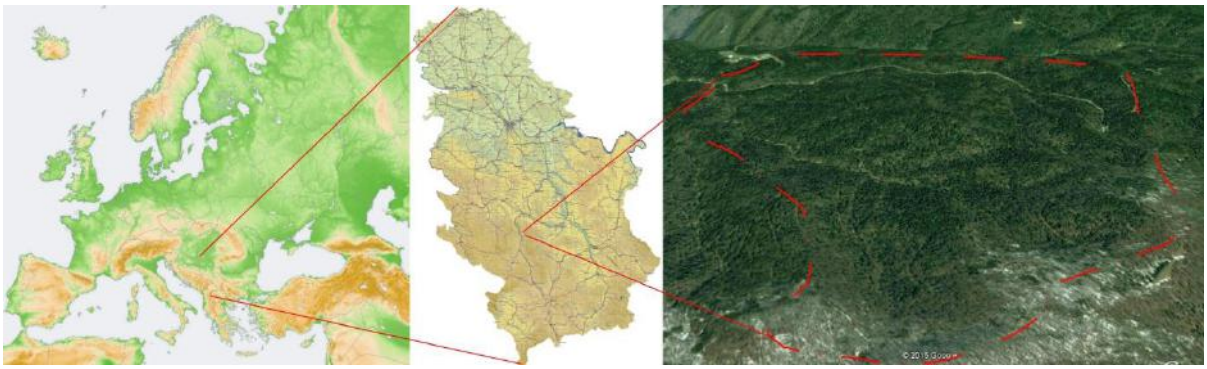
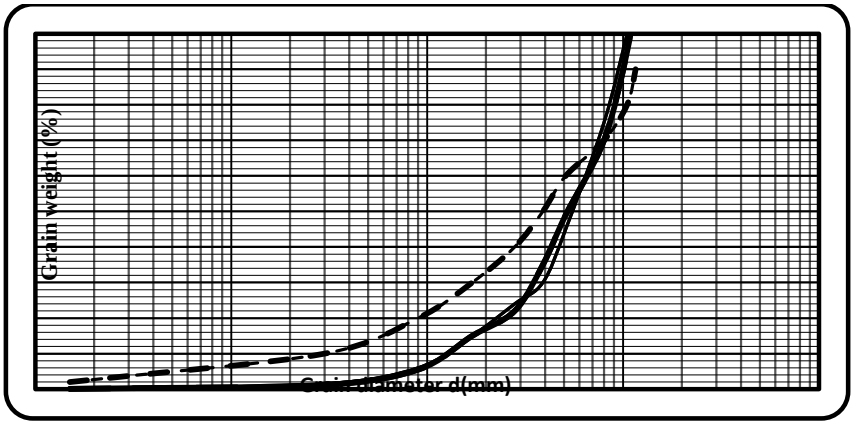


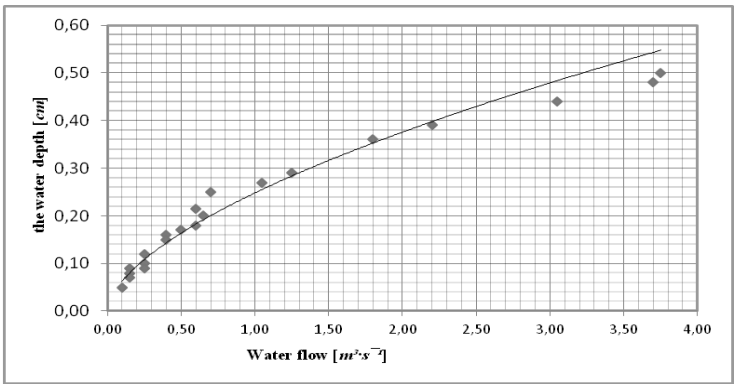
Figure 1. The study area of the Teaching Base of the Faculty of Forestry from Belgrade, the river Gvozdacka Reka on Mt. Goc, the Zapadna Morava River basin.

The rocks of groups 2) and 4) account for 80.23% of the catchment area and play the decisive role in the supply of river sediment in the upper, middle and downstream parts of the stream and river valley. In the downstream reaches, bed material consists of coarse gravel and sand fractions which are are transported as bedload and partly as suspended load during floods.
In norder to measure particle size distribution, bulk density, and specific weight of sediment, samples were taken from the river bed and banks (Fig. 5.)
The particle size distribution for fraction sizes > 0.1mm was obtained through standard sieving method and the results are graphically reported in Equation 1. and in Table 1. presenting the analysis of sediment movement at different discharge rates.
Further analysis consists in the calculation of a few characteristic diameters such as D_{10}, D_{50} and D_{90}, i.e. the dimeter by which 10, 50 and 90% of the distribution is finer. The sampled sediment grain diameters and weights were subject of hydraulic analysis.



Equation 1. Diagram of the particle size distribution of the sediment from the river Gvozdacka Reka. (Source: original)

To determine the discharge at the selected cross section (Fig. 4), the following measurements were made: cross section area and flow velocity, by using a current meter along different verticals, to obtain mean cross-section discharge. Based on several measurements in different hydrological conditions, we defined the flow rating curve (Fig. 2).



Equation 2. The Gvozdacka Reka discharge curve at the intake for fishpool. (Source: original)

The river discharge was measured in different seasons and weather conditions over the period 1993 - 2006 (Fig. 2). During the same interval, flood waters were sampled for the measurement of suspended sediment concentration.

The threshold conditions for bedload entrainment were calculated using Meyer-Peter Muller's formula; i.e. for the calculation of critical tractive forces. For different sizes of eroded material, the calculation of different phases of initiation of motion and also the definition of the method of transport through the catchment was performed using the comparison of tractive forces with critical tractive forces.

In this sense, the following methods were applied:

Modelling the complete physical process of eroded material movement under the effect of the river hydro-dynamic forces;

Field methods, including the survey of water discharge in the main stem and the tributaries;

Sediment sampling for the measurement of particle size distribution, bulk density, and specific weight;

The samples were analysed for other geomechanical characteristics, the angle of internal friction of sediment and cohesion;

The morphology of natural channel, cross sections, stream bed slope, and the slope of the water surface were defined based on field geodetic surveys;

Hydrometric measurement of water discharge at the sampling point;

Samples of torrential water for psammological research and estimation of suspended sediment concentration.

Particle movement (suspended sediment and bedload) is directly related to sediment grain sizes and particle specific weight and also to the stream turbulent regime and tractive forces in the stream. Critical tractive forces, according to Shields' criterion (1936) are defined depending on sediment specific weight, water density and median particle diameter.

$$\tau_{cr} = 0.047 \cdot g \cdot (\rho_s - \rho) d_{sr} \, [\mathrm{kN/m^2}] \qquad (1)$$

where:

τ_{cr}, critical tangential stress;

g, acceleration of gravity;

ρ_s, sediment density;

ρ, water density;

d_{sr} (design) of particle diameter

In eq. (1) the dimensionless Shields' parameter was take as 0.047, rather than the original value of 0.056, au suggested by Andrews (1983), because of the coarsenees of bed material.

In this study, attention was focused on the conditions of particles entrainment from river bed and banks, i.e. on the assessment of the conditions under which some sediment fractions start moving as bedload.
Tractive force is defined by the equation:

$$\tau_0 = \rho_v \cdot g \cdot R \cdot J \text{ [kN/m}^2 \tag{2}$$

where:

τ_0, tangential stress;

g, acceleration of gravity;

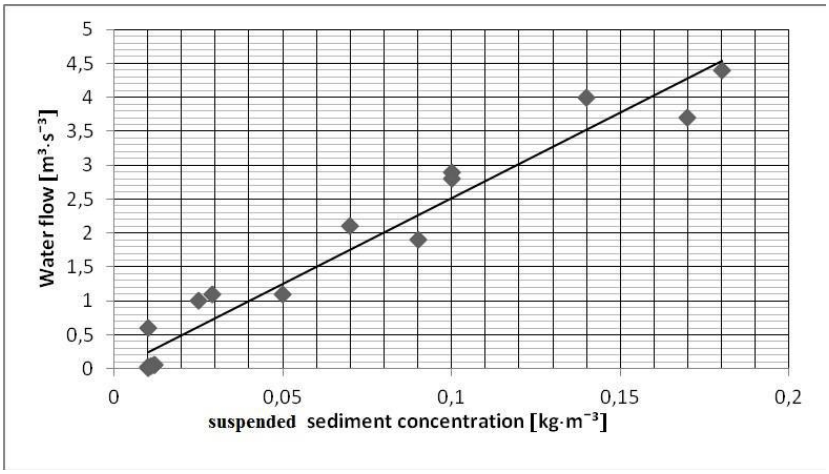
ρ_v, fluid density;

R, hydraulic radius;

J, slope of the water surface.

Results and Discussion

Suspended load



Equation 3. Suspended sediment concentration in the function of discharge. (Source: original)

Equation 2 shows the variation of suspended sediement concentration with discharge. The correlation between these two variables is rather high since flow discharge explains 95% of suspended load conscentration.
Maximum oncentrations, as high as 0.193kg·m^{-3} were measured with a discharge (Q) of 4.4m^3·s^{-1}. During 2006, some considerably higher concentrations of suspended sediment were recorded in coincidence with particular hydrological conditions (Fig. 5). These conditions were caused artificially by water releasing from the reservoir on the Prerovska Reka and, on such occasion, part of the infilled sediment was moved from the storage (Fig. 5).

Bedload transport

The transport of large fractions of bed material occurs periodically, that is mainly during floods. Depending on the mode of transport, river sediment is classified as bedload in the form of sliding and rolling grains and suspended load. Tangential stresses occur throughout the stream wetted perimeter, and the highest tangential stresses occur at the deepest places in the stream (Lajczak, 1996).

Figure 4. The River Gvozdacka Reka during the discharge Q=2.49$m^3 \cdot s^{-1}$ (Photo: Djekovic)

Figure 5. Deposited organo-mineral sediment behind the dams (Photo: A. Andjelkovic)

Table 1. Measured discharges in the main stream and its tributaries (10-12). 05.2010

Prerovska Reka	**Bela Reka**	**Gvozdacka Reka**
Q (m^3sec^{-1})	**Q (m^3sec^{-1})**	**Q (m^3sec^{-1})**
0.110	0.120	0.230
0.219	0.198	0.417
0.215	0.235	0.450
0.215	0.785	1.000
0.215	1.785	2.000
0.215	2.285	2.500

It is known that the rate of flow next to the stream bed equals zero, due to water viscosity and boundary roughness, but it changes rapidly at a small distances from the stream bed. As the rate of flow next to the stream bed is the rate of flow which occurs at the distance of *0.04h* from the stream bed, the rate of flow next to the stream bed is calculated in relation to the mean flow rate.

$$V_0 = V_p \cdot \eta^k = 1.20 \cdot V_{sr} \cdot 0.04^{0,20} = 0.63 \cdot V_{sr}(m^3 \cdot s^{-1}) \quad (3)$$

where:

V_0, velocity near the bottom (0,04h);

V_p, speed on the water surface;

V_{sr}, the mean flow velocity profile;

η^k, the ratio of the surface velocity and the velocity near the bottom.

Threshold force is the force, at which sediment grains of certain sizes and bulk density are on the brink of being entrained. The larger the sediment, the greater force is necessary to entrain the sediment. As tangential stress is related to flow depth and the energy slope, assumed equivalent to the water surface slope (and consequently to streambed gradient) for very low gradients, and as these parametrs depend on discharge, it is not only sediment transport competence of the stream that can be determined at each discharge in the river channel, but also the grain sizes which can be entrained in such conditions.

In the study reach, as bedload is moved mainly in flood waves and he study was based on Shields' criterion defined by Eq (1). This equation was derived from flume experimental measurements entarinment of particles with a given mean diameter. Eq. (1) was used to calculate the critical tractive force for grain diameters spanning from D_{10} to D_{90} of the study

site bed material. The comparison of tangential stresses with critical stresses can be applied in the determination of the phases of the initiation of motion of individual grain sizes in different hydrological conditions. A straight, 250 m long study reach (Fig. 6.) was selected downstream of the storage intended for fishpool (Fig. 6).

Morphological analyses included topographic surveying of the study reach consisting in the measurement of cross sections and longitudinal profile. The cross sections areas are uniform throughout the reach, with depth ranging from 21 - 77 cm, while the width of water surface at maximum discharge amounted to about 3.80 m. The streambed gradient was found to be J = 0.0229 (Billi, P., 2011) equal to the slope of the water surface. Graduated rods were used for water depth measuring during floods.

To ensure the general stability of the watercourse, the banks are protected with large stone blocks D=500-1000 mm, which could not be move by dicharges much higher than the largest flow measured. The field data indicate that the size and the bed slope did not change and other hydraulic parameters, such as sediment sizes and the resistance coefficient, remained constant. The discharge during water release form the storage was determined using the flow rating curve obtained for the study cross sections.

As the simulation of flood wave discharges in this stream reach can be repeated several times per day by releasing water from the storage, the experiments are very significant for the region of hill and mountain streams. Based on the grain-size distribution of bed material in the Gvozdacka Reka, the calculated sediment bulk density is as follows:

$$\rho_s = 2.054\ t.m^{-3}$$
$$\tau_0 = 0.047 \cdot g \cdot (\rho_s - \rho) d_{sr}$$
$$(\rho_s - \rho) = 1.054 N \cdot m^{-3} \quad (5)$$

The tractive force in the stream is defined by the equation:

$$\tau_0 = \rho_v \cdot g \cdot h_{\max} \cdot J \quad (6)$$

Table 2. Thresholds of sediment entrainment in the Gvozdacka Reka

Discharge (Q) ***l·sec***	**Design sediment grain diameter in [mm]**									τ_{cr}	τ_o	**Water depth**
	d10	***d20***	***d30***	***d40***	***d50***	***d60***	***d70***	***d80***	***d90***	kN/m^2	kN/m^2	***M***
125	0.09-3									0.0000437-0.0015	0.0472	0.21
216		5.5-9								0.0026-0.0043	0.0539	0.24
230			15-17							0.0073-0.0083	0.0564	0.25
417				22-28						0.0107-0.0136	0.0808	0.36
450					30-39					0.0146-0.0189	0.0819	0.36
1000						45-51				0.0219-0.0249	0.1213	0.54
2000							70-80			0.034-0.0389	0.1582	0.70
2490								95-105		0.0462-0.0510	0.1703	0.76
2500									116	0.0563	0.1724	0.77

Based on the above analysis, Table 2, at the discharge Q=2.5m$^3 \cdot$s^{-1}, all fractions of sediment from the channel of the river Gvozdacka Reka will be moved and only coarse particles larger than 116 mm will remain. By comparison of true tractive forces with critical tractive forces, it can be observed that tractive forces are higher than critical forces within the analysed discharge range in thestudy reach. Therefore, conditions for the entrainment ofa large

proportion of bed material are expected to occur commonly, resulting in the morphological changes in the stream cross section. Sediment grain diameters d<116 mm are transported under the discharge rates Q>0.230 $m^3 \cdot s^{-1}$, mainly as bedload. In such conditions, water depth ranges from 210 to 250 mm.

The total volume of the reservoir after the construction of the dam was W = 2730 m^3. After 10 years' of filling the reservoir, reservoir volume was decreased by 70% and amounted to W = 820 m^3; of which 1910m^3 of organo-mineral deposits.

Morphological analysis of the river channel

Figure 6. The reservoir from which water was released, on the experimental stream reach (*Photo: A. Andjelkovic)*

Conclusions

Sediment transport in the experimental stream reach of the Gvozdacka Reka, as well as the changes in morphology of the stream profile, were measured by successive releases of water from the storage (Fig. 5). The following conclusions were made based on the above research:

Based on the above research, by implementing the same methodology, it is possible to define the type of material for the revetment and the construction of the specific types of river improvement structures. Also, it is possible to calculate the entrainment thresholdsfor different particle sizes, as well as the phases when and under which conditions it is necessary to protect the stream channel against erosion processes caused by river water.

This research is unique because it is done on a natural model, filling and emptying the reservoir during the day. This is achieved by simulation of flood wave downstream from the reservoir. Maximum flows were to 2.49 $m^3 s^{-1}$, and the maximum water flow of samples was taken by the flood waters, by the laboratory methods it was identified the concentration of suspended sediment load transport.

The results of these studies have pointed to the importance of the impact of land use patterns, as well as the impact of anthropogenic factors on the intensity of ssoil erosion. Although the river basin is covered with the 95% of beech-fir forests, accumulation was feeded with 70% sediment of organomineral origin, for a period of 10 years.

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INFLUENCE OF DIFFERENT PLANTING CONDITIONS ON THE GROWTH OF SPRUCE FOREST CULTURES (CASE STUDY: SIVINSKII FOREST DISTRICT, PERMSKII KRAI)

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Abstract

This article is discussion of research results of spruce forest cultures carried out in summer 2014. Investigations were carried out in western part of Permskii krai (Russia). Forest cultures were planted on cutover areas of spruce shamrock and herbaceous forests in 2008, 2011 and 2014. Soils of cut over areas are damp (C3) and normal (C2) loam. The influence of soil treatments by two kinds of forest ploughs (PL-1 and PLP-135, made in 2013) on growth of four-year seedlings was studied. The width of planting place profiles after using forest ploughs was measured. The soil density at furrow and ridges of planting places was measured. A planting place ramping was defined. The annual growth of spruce forest cultures and their conservation was measured. Also, the growth of spruce forest cultures planted after soil treatment by forest plough “PL-1” on cutover areas on different forest site was studied. The two- and four-year seedlings were planted. The width of planting places created by ploughs “PL-1” and “PLP-135” differ from literature data. The practical width of furrow after soil treatment by “PLP-135” is 105 cm instead of 135 cm. The practical width of furrow after soil treatment by “PL-1” is 54 cm instead of 1 meter. The density of furrow soil (1.9-2.0 g/cm3) exceeds optimal data for spruce. The use of the forest plough “PL-1” does not provide furrow clearness from grasses in planting year. The annual growth of spruce cultures did not depend on the height of seedlings in 2014. Two-year seedlings planted into furrow have the better growth and conservation than four-year seedlings. On damp cutover area, the forest cultures must be planted into ridges. The plough “PLP-135” is the best for ridge creating.

Keywords: *artificial reforestation, spruce forest culture, forest plough, profile of planting place, forest site.*

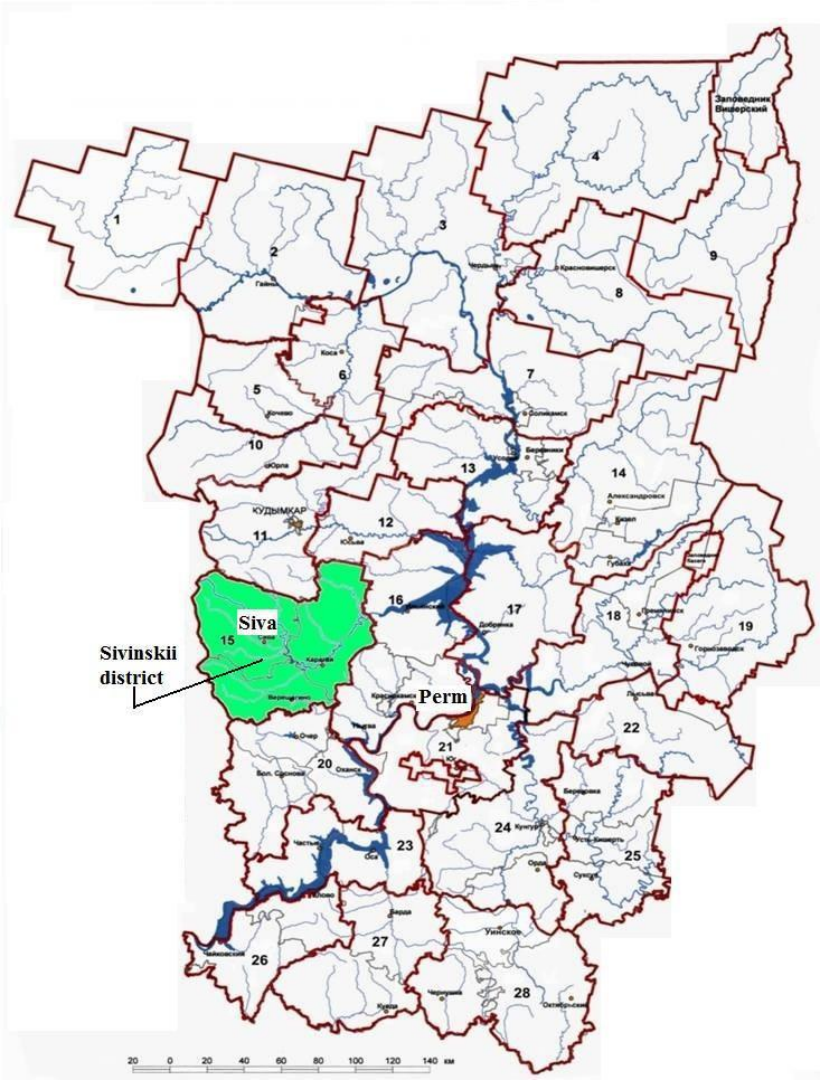
Introduction

Percentage of Permskii krai forest land is about 62 %. Annual deforestation area is 25000 ha. The Forest Code of the Russian Federation provides the forest inexhaustible using and up to date reforestation with valuable wood species. These are coniferous species for Permskii krai. Quantity of annual precipitations changes are from 500 to 1000 mm. Such conditions promote natural reforestation. Also these conditions are favorable for growth and development of vegetation layer. Grass crowd out stand or does not let grow of self-seed crop on plots with rich soil. In this case we use artificial reforestation. The artificial reforestation in Permskii krai is about 5500 ha per year. It is 20 % of entire area of reforestation (Indicators, 2014). Forest ploughs are the main soil cultivation tools for artificial crops. They are cheap and easy for exploitation than tools with active work details. The soil cultivation by rip dozer is effective on the clearance of big quantity stamps, but it reduces soil fertility. In the seventies of the 20th century perm forest scientists (as other scientists of USSR) studied different ways of soil cultivation for artificial crops. They discovered the soil structure differences after cultivation. They used forest ploughs (PKL-70-1, PKL-70-2, PLP-135) and rip dozers (D-210 V, D-259). Also our forest scientists studied spruce planting into furrow and ridge created by forest plough. But those works were not finished. Now our foresters continue to create forest

crops using the worst way (in most cases). They usually plant spruce and pine seedlings into plough furrow. Our work is showing negativity of this practice.

Material and methods

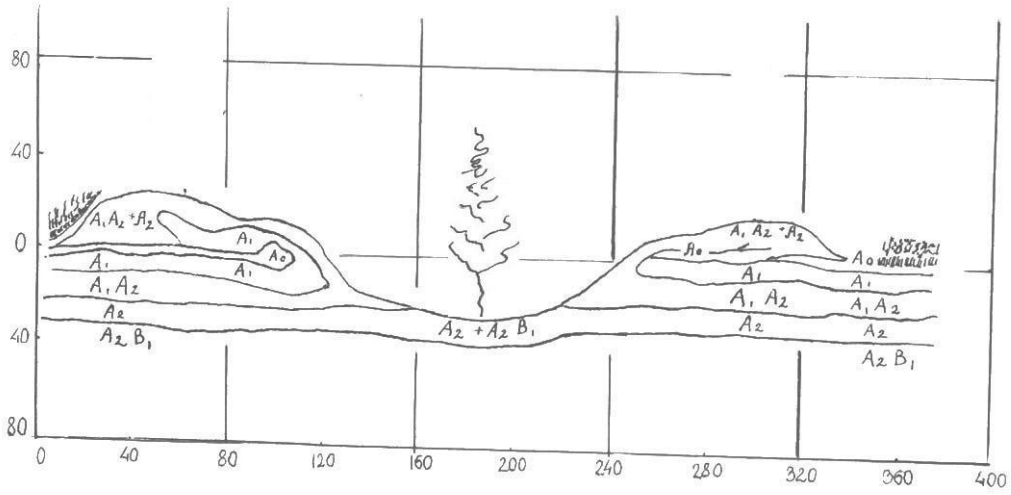
Research was made on the territory of Sivinskii forest in Permskii krai (pic. 1) in summer 2014. The forest district is in the plain part of Permskii krai. The territory has plain-mountain relief. Forest crops on cutover areas in spruce shamrock and herbaceous forests were analyzed. These forests were felled in 2008, 2011 and 2014. Soils of cutover areas have a high fertility (the soil is loamy), but differ in moisture conditions. The spruce shamrock forest has a normal moisture condition. This forest is C2 of forest site type by Pogrebniak classification (Sennov S.N.,2011). The spruce herbaceous forest has a high moisturization. This spruce herbaceous forest responds to C3 of forest type by Pogrebniak classification. The aim of the research is developing recommendations for forest crop creating under the condition of Sivinskii district. The program of the research includes three parts. The first is influence of soil cultivation with different forest ploughs on growth and development of spruce forest crops. The second is influence of different forest site type on growth and development of spruce forest crops. The third is age influence of spruce seedling on growth and development of spruce forest crops.



Picture 1. – Placement of forestry districts on Permskii Krai territory

The tillage was carried out by forest ploughs PL-1 (forest plough, 1 m of width) and PLP-135 (). The plough PL-1 was aggregated with tractor «LHT-55» (forestry tractor, power is 55 h.p.). Such aggregate cannot root out stamps and should route away them making ruptures in furrows. The plough PLP-135 was aggregated with tractor T-130. This aggregate has grubber and make unbroken furrows for forest crop planting. The comparison of different ploughs was carried out on cutover area of spruce shamrock forest. Spruce artificial crops were planted into furrow as four-year seedlings. The efficacy of artificial reforestation of different type of forest site was carried out on forest crop plots planted after plough PL-1 using. Planted seedlings were two-year and four-year. Seedlings were planted into furrow with spacing 0.7-0.9 m.

The research program consisted of four sections. The first is the measurement of the cross section of planting space created with forest ploughs. The second is defining soil density of furrow and ridges (pic. 2). The soil density was measured by Nekrasov's soil borer. The third is the measurement of occlusion with herbaceous vegetation at planting places. The fourth is the measurement of the spruce forest crop growth. Full heights of spruce forest crops, annual apical growth, stipes thickness and placement of spruce crop relative to each other were measured. The capacity for survival of artificial crop was defined.



Picture 2. - Cross section of planting space created by PLP-135

Results and discussion

The plough PLP-135 makes unbroken planting furrow. Ruptures in planting places after using plough PL-1 are 13 % at their length (table 1). The depth of furrow is equal. The furrow soil density is equal, too. The density of furrow soil (1.9-2.0 g/cm3) exceeds optimal data for spruce. The width of planting places created with ploughs "PL-1" and "PLP-135" differs from literature data. The practical width of furrow after soil treatment with "PLP-135" is 105 cm instead of 135 cm. The practical width of furrow after soil treatment by "PL-1" is 54 cm instead of 1 meter.

Table 1. – The description of planting places for spruce artificial crop (cultivating – June 2013, planting – May 2014, study – August 2014)

Plough	Percentage of furrow ruptures, %	Width of cultivated stripe, cm	Width of furrow, cm	Depth of furrow, cm	Furrow soil density, g/cm^3
PL-1	13	194±9	54±4	14±1	2.0±0.3
PLP-135	0	302±13	105±3	16±3	1.9±0.3

Cutover areas (forest type “spruce shamrock forest”) were overgrown actively with ashweed (*Aegopodium podagraria* L.), blackberry (*Rubus caesius* L.), wild strawberry (*Fragaria vesca* L.) and starwort (*Stellaria holostea* L.). Orobus (*Orobus vernus* L.) and tufted vetch (*Vicia cracca* L.) were less than the others.

On the plot with cultivating with plough PLP-135 there are Adam's-rod (*Verbascum thapsus* L.), bottlebrush (*Equisetum sylvaticum* L.) and yellow bedstraw (*Galium verum* L.). On the plot cultivated with plough PL-1 there are Canadian thistle (*Cirsium arvense* L.), dog nettle (*Galeopsis ladanum* L.), white dead-nettle (*Lamium album* L.). The planting furrow was overgrown with forest grasses more intensive after cultivating with plough PL-1. The projective cover of grasses on furrow was from 30 to 60 %. The most part of the overgrown land was made by annual meadow grass (*Poa annua* L.) and blackberry. Often we saw sprouts of Canada fleabane (*Erigeron canadiensis* L.), germander speedwell (*Veronica chamaedrys* L.) and Canadian thistle. Seldom there was bottlebrush on the furrow. At the same time the ridges were overgrown more intensive (the projective cover of grasses was from 40 to 100 %). Ashweed occupied the most part of ridges.

Three years later after tree cutting in forest type “spruce shamrock forest” the living ground cover was accompanied with bush grass (*Calamagrostis epigejos* L.) and rosebay willow-herb (*Chamaenerion angustifolium* Scop.). At the same time the cutover area in forest type “spruce herbaceous forests” was overgrown with European aspen (*Populus tremula* L.).

Created with plough PLP-135 furrow was overgrown with blackberry and bottlebrush. Adam's-rod sprouts appeared less often. The projective cover of herbaceous vegetation was less than or equal to 5 %. At the same time the projective cover of herbaceous vegetation was from 10 to 70 % on ridges. Ridges were overgrown with Adam's-rod, starwort and blackberry. Sometime there were strawberry, ashweed and sparrow-tongue (*Poligonum aviculare* L.).

Planted in 2014 spruce seedlings had a planting stress. However, annual apical growth of spruce forest crop planted after plough PL-1 was more at 1.2 time than after plough PLP-135 (table 2). The conservation of spruce artificial crop created after plough PL-1 was 10 % lower than the second variant. The espacement of seedling into furrow created with plough PL-1 did not allow realizing a plan for optimal planting density (2652 instead 3500-4000 off/ha). At the same time, planting density was 3580 off/ha after using PLP-135. The annual growth of spruce cultures did not depend on the height of seedlings in 2014.

Table 2. – Consistence of spruce artificial crop (cultivating – July 2013, planting – May 2014, study – August 2014)

Tool	Width of inter-row spacing, m	Planting spacing, m	Planting density, off/100 long meter	Conservation of forest crop in 1 year, %	Apical growth, cm
PL-1	5-7	0.59	156	81	3.1±0.3
PLP-135	4-6	0.57	179	91	2.6±0.2

Also, we studied spruce artificial crop growth in different forest type (spruce shamrock and spruce herbaceous forests). Soil cultivating was made with plough PL-1. Study results of artificial crop planted as 4-year seedlings are in table 3. The consistence of artificial crop planted as 2-year seedlings are in table 4. Two-year seedlings planted into furrow have the better growth and conservation than four-year seedlings.

Table 3. – Annual apical growth of spruce artificial crop in different forest type (cultivating – July 2011, planting – May 2012, study – August 2014)

Forest type (type of forest site)	Apical growth, cm			Conservation of forest crop, %
	in 2012	in 2013	in 2014	
Spruce shamrock forest (C2)	4.9	6.9	8.1	37.6
Spruce herbaceous forest (C3)	4.5	6.9	6.4	28.6

Table 4. – Consistence of spruce artificial crop planted by 2-year seedlings (cultivating – July 2007, planting – May 2008, study – August 2014)

Forest type (type of forest site)	Conservation of forest crop, %	Butt diameter, mm	Height, cm
Spruce shamrock forest (C2)	94	11.1±0.5	61±2
Spruce herbaceous forest (C3)	85	9.6±0.7	49±3

Many authors believe artificial crop must planted into ridges as in condition C3 as in condition C2 (Merzlenko, M.D., 2002, Redko G.I., 2008, Shutov I.V., 2007). Study results of ridges created by ploughs PL-1 and PLP-135 are in table 5. Data of table show ridges after PL-1 do not provide optimal width of ridge. The ridge can dry. Other authors wrote about it.

Table 5. – Characteristics of ridges created forest ploughs (cultivating – July 2013, study – August 2014)

Tool	Width of ridge top, cm	Height of ridge, cm	Soil density of ridge, g/cm^3	Soil density of raw piece of land, g/cm^3
ПЛ-1	30±4	12.6±1.6	1.15±0.2	1.1±0.2
ПЛП-135	53±8	9.0±2.6	1.6±0.2	1.2±0.3

The ridge after PLP-135 is better for artificial crop planting than ridge created by PL-1. However, acceptable width of ridge is in evidence about 54 % of cultivated places and soil density of such ridge is not optimal for spruce growth (1.6 instead 1.3 g/cm^3, this is maximal acceptable value (Merzlenko M.D, 2002).

Conclusions

Forest ploughs are used extensively for the soil cultivating as to plant artificial crop in many forestry districts of Permskii Krai.

Planting furrows created with forest plough PL-1 are overgrown living ground cover to 60 % of surface in planting year. The furrow soil density is 2 g/cm^3. This is more optimal value in 1.7 time.

Planting furrows after cultivating with forest plough PLP-135 are overgrown living ground cover to 5 % of surface. Furrow soil density is unsuitable for spruce growing too and one is 1.9 g/cm^3.

The apical growth spruce planted after plough PL-1 is 1.2 times more than after cultivating with plough PLP-135.

The growing of spruce artificial crop in condition forest type “spruce shamrock forest” is more from 1.1 to 1.2 times than such data for condition “spruce herbaceous forest”.

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QUANTIFICATION OF THE STAND STRUCTURE TO DEFINE THE ECOSYSTEM ALPHA DIVERSITY: A CASE STUDY IN A BEECH STAND

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Abstract

The structure of high beech stand in the Eastern Serbia was quantified using different structural indices, in order to assess the ecosystem alpha diversity. For quantification of the simple structure, applied were the Lorenz index, and for the spatial structure applied were the aggregation index, while for description of the structure on small areas - the indices of individual trees based on the relations of neighbouring trees - uniform angle index, mean directional index, diameter differentiation index, and dominance index were used. Lorenz index and diameter differentiation index show that it is well structured stand, both over a small area and at the level of the whole stand. Aggregation index, uniform angle index and mean directional index provide random arrangement of tree positions within the stand with a mild tendency to regular tree pattern. Dominance index shows that 53 % of the nearest neighbours of an observed tree have a larger diameter. The greatest advantage of the application of modern indices for structural quantification is that the different aspects of stand structure can be expressed numerically, which is opposing to classical descriptive structure presentations. Due to the pronounced structural diversity of beech forests in Serbia application of these indices is necessary in order to objectively analyze the structural changes over a longer time.

Keywords: *simple structure, spatial structure, ecosystem diversity, beech, index.*

Introduction

Structure refers to a specific arrangement of elements in a system. Accordingly, forest structure, describes the distribution of tree attributes (characteristics) within a forest (von Gadow, 2005). The description of forest structure defines the percentage of certain attributes within a population, e.g. the percentage of tree species or the percentage of different tree dimensions (Vanclay, 1998).

In the widest sense, forest structure is described as simple and spatial. Simple structure denotes the frequency distribution of individual tree attributes, without knowing the spatial position of trees. Spatial structure describes the distribution of tree attributes taking into account the inter-neighbour relations between individual trees.

In the management of natural (primarily structured and mixed) forests, the study of forest structure has decisive significance in the assessment of forest condition, analysis of the previous management and definition of future management methods.

Also, in natural forests, structure is the main and most often the only measurable indicator in the determination of species and ecosystem diversity (Pommerening, 2002). Species diversity has become a very important aspect of forest management and conservation. It can be described using numerous parameters (Aquirre et al., 2003). At the spatial level, ecosystem diversity is divided into alpha, beta, gamma and delta diversity (MacArthur, 1965). In forest ecosystems, alpha diversity represents the diversity within a forest stand, beta diversity refers to the differences between forest stands, and gamma and delta diversities refer to wider

contexts (Lähde et al., 1999). Nowadays, it is especially relevant to know alpha diversity. Its determination requires the study of spatial distribution of trees and their attributes (Ferris, Humphrey, 1999).

Previous researches were mostly devoted to simple or basic stand structure. Simple structure consists of: diameter structure, height structure, species diversity, coefficient of homogeneity, distribution of basal area, volume, volume increment, crown length, etc.

Compared to simple structure, the study of spatial stand structure is a more complex problem. The quantification of spatial structure is based on a number of indices (parameters).

Firstly, the aim of this study was to quantify the structural diversity of a beech stands, using various structural indices, aiming at an objective definition of the ecosystem alpha diversity. Secondly, to show the possibilities of practical application of these indices in beech forests in Serbia.

Materials and methods

Study area and data collection

The study area is a high stand of beech in Eastern Serbia (stand 33a, management unit “Majdan-Kučajna“). Stand area is 22.7 hectares. Its latitude 44°24'05", longitude 21° 36'32", altitude is 410-520 m, slope 7-28 degrees. The prevailing aspect is north-west. Parent rock consists of dense limestones, and the soil is cambisol eutric, depth 40-80 cm. The stand is classified as a submontane forest of beech (*Fagenion moesiacae submontanum* B.Jov.1976). By silvicultural and structural form, it is a high group-selection uneven-aged stand of beech, with virgin forest characteristics. Site class is II, conopy closure is 0.90, percentage of beech in the volume 97%, stand quadratic mean diameter is 39.4 cm, and Lorey’s mean height 31.0 m. There are 274 trees per hectare, basal area is 33.4 m^2/ha, volume is 522.5 m^3/ha, and current annual volume increment is 8.6 m^3/ha. (Koprivica et al., 2008; Matović, 2012).

Simple circular sample plots of 500 m^2 (radius 12.62 m) were established (total 23), distributed in square grid network design, spacing 100 m (a circle represents a hectare). Diameter and height of all trees above taxation limit (>10 cm) on sample plots in the stand were measured. Also, to research the spatial structure, the data recorded for every stem on each plot included the azimuth and the distance from plot centre.

Indices for quantification of stand structure

The quantification of simple structure was performed using the Lorenz index (Lee et al., 1999). The aggregation index was used for the quantification of spatial structure (Clark, Evans, 1954). Indices of individual trees based on inter-neighbour relations between individual trees were used for the description of structure on small areas (uniform angle index, von Gadow et al., 1998; mean directional index, Corral Rivas, 2006; diameter differentiation index, Füldner, 1995; dominance index, Hui et al., 1998). The Lorenz index was calculated from the field data in Excel and Statgraphics, and the indices of spatial structure were computed using an especially developed software package Crancod (Pommerening, 2006).

The Lorenz index. The Lorenz index *L* numerically defines the plot or stand simple structure. A measure of stand homogeneity can be the tree diameter, basal area, volume, etc.

Lee et al. (1999) calculated the Lorenz index from the basal area percentage and the percentage of tree number. Trees are first ordered from the thinnest diameter in the increasing order, then basal area is calculated for each tree. The basal area percentage pg_i is calculated from the thinnest-diameter tree to the i^{th} tree using equation (1). Percentage of tree number pn_i to the i^{th} tree is calculated using equation (2).

$$pg_i = \frac{1}{G}\sum_{j=i}^{i} g_j \text{ , } G \text{ - total basal area} \qquad \text{(Eq.1)}$$

$pn_i = \frac{i}{n}$, n - total number of trees (Eq.2)

Lorenz curve is graphically best represented as a power function $pg_i = pn_i^{\beta}$. β can be equal to or greater than 1. When β =1, the stand is fully homogeneous, and with the increase in the coefficient β, the stand structure heterogeneity also increases.
The Lorenz index of sample plot structure is calculated using equation (3).

$L = 1 - \frac{1}{\beta}$, $0 \leq L < 1$ (Eq.3)

From the aspect of basal area, fully homogeneous stand is when L = 0, but with the increase in L value, the stand becomes structurally heterogeneous.
The Lorenz index of stand structure is calculated as an arithmetical mean of sample plot indices (4).

$\bar{L} = \frac{1}{n}\sum_{i=1}^{n} L_i$, i = index of a sample plot (i=1..n) (Eq.4)

Aggregation index. Aggregation index *CE* describes the spatial structure and represents the relation between the average measured distance between a tree and its nearest neighbour $\bar{r}_{obs}$ and the average distance when the trees are randomly distributed $\bar{r}_{\exp}$ in a sample plot or stand (5).

$CE = \frac{\bar{r}_{obs}}{\bar{r}_{\exp}}$ (Eq.5)

The average distance between a tree and its nearest neighbour $\bar{r}_{\text{obs}}$ in a sample plot is calculated as the ratio of a sum of distances between all trees and their nearest neighbours and the number of trees N (6). The average distance when the trees in the stand are randomly distributed $\bar{r}_{\exp}$ is calculated using equation (7).

$\bar{r}_{obs} = \frac{\sum_{i=1}^{N} r_i}{N}$ (Eq.6)

$\bar{r}_{\exp} = \frac{1}{2\sqrt{\frac{N}{A}}}$, where A is the sample plot or stand area. (Eq.7)

Theoretically, *CE* ranges from 0 (when the trees are clumped) to 2.1491 (when the tree pattern is regular in the hexagonal grid). *CE* = 1, represents a random arrangement of tree positions (Poisson distribution) in the sample plot or stand.
One of the problems occurring in the research of spatial structure is the estimation of distances between edge trees in the sample plot or stand and their nearest neighbours. This problem is solved using edge-correction. In the study stand, the spatial structure was studied based on the of edge correction method *NN1* (Pommerening, 2006).
Indices of individual trees based on the relations of neighbouring trees for the description of the structure on small areas. These indices describe the spatial structure using the structural groups of 4 neighbours in relation to reference tree *i* (*Figure1*).

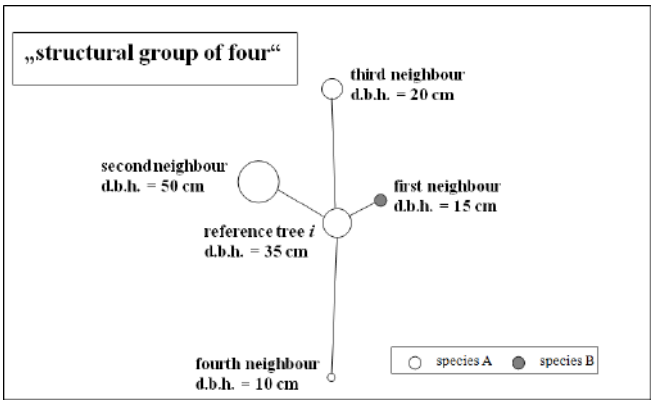

Figure 1. *Structural group of 4 neighbours in relation to the reference tree i (Pommerening, 2002)*

The uniform angle index W describes the degree of regularity of spatial distribution of four nearest trees in relation to the reference tree *i*. *W* is calculated from the ratio of true values of angles between 4 neighbouring trees α_j and a predefined standard angle α_o. Binary random variables v_J are calculated by comparing each value of α_j with standard angle α_o. Uniform angle index is defined as the ratio of angles α_j between four neighbouring trees which are greater or lower than the standard angle α_o (8).

$$W = \frac{1}{4}\sum_{j=1}^{4} v_j \text{, where } v_j = \begin{cases} 1, & \alpha_j < \alpha_o \\ 0, & \alpha_o < \alpha_j \end{cases}, \; 0 \le W \le 1 \qquad \text{(Eq.8)}$$

The uniform angle index of sample plot $\overline{W}$ is calculated as the arithmetical mean of individual values of *W* for all trees in the sample plot.
Hui, von Gadow (2002) determined 72° as the optimal standard angle, when the trees are randomly distributed at the value $\overline{W}$=0.5. When $\overline{W}$<0.4815, the neighbouring trees tend to regular distribution, and when $\overline{W}$>0.5130 the trees are clumped in relation to the reference tree.
Mean directional index R is defined as the sum of the unit vectors from the reference tree *i* to its *n* nearest neighbours (9).

$$R = \sqrt{\left(\sum_{j=1}^{n} \cos\alpha_{ij}\right)^2 + \left(\sum_{j=1}^{n} \sin\alpha_{ij}\right)^2}, \qquad \alpha_{ij} \text{ is the angle between trees } i \text{ and } j \text{ and a reference direction} \qquad \text{(Eq.9)}$$

Mean directional index of a sample plot $\overline{R}$ is calculated as the arithmetical mean of individual values of *R* of all trees in sample plot.
When $\overline{R}$=1.799, the neighbouring trees are randomly distributed, if $\overline{R}$=0 trees are regularly distributed in square grid, and if $\overline{R}$>1.799 trees are clumped in relation to the reference tree.
Differentiation index T describes the spatial distribution of tree dimensions (diameter at breast height, height, etc.). Diameter differentiation index, when the structural group of 4 neighbours is applied, is calculated using equation (10).

$$T = 1 - \frac{1}{4}\sum_{j=1}^{n} \frac{\min\left(DBH_i, DBH_j\right)}{\max\left(DBH_i, DBH_j\right)}, \qquad j = 1 \ldots n \text{ (4) neighbour trees, DBH – diameter at breast height} \qquad \text{(Eq.10)}$$

Diameter differentiation index of sample plot $\overline{T}$ is calculated as the arithmetical mean of individual values of *T* for all trees in the sample plot.

Low differentiation is when $0.0 \le \overline{T} \le 0.3$, medium $0.3 \le \overline{T} \le 0.5$ strong $0.5 \le \overline{T} \le 0.7$, and very strong $0.7 \le \overline{T} \le 1.0$.

Dominance index U quantifies the percentage of neighbouring trees having larger dimensions (diameter at breast height, height, etc.) than the reference tree *i*. When applied to a structural group of 4 neighbours, diameter dominance index is calculated using equation (11).

$$U = \frac{1}{4}\sum_{j=1}^{n} v_j \ , \ v_j = \begin{cases} 1, & DBH_j \ge DBH_i \\ 0, & DBH_i \ge DBH_j \end{cases} \qquad \text{(Eq.11)}$$

When *U*=0, it means that all neighbouring trees have a smaller diameter, when *U*=0.5 three neighbours have smaller diameter and three neighbours have larger diameter, and when *U*=1.0 all neighbouring trees have larger diameter than the reference tree.

Diameter dominance index in the sample plot $\overline{U}$ is calculated as the arithmetical mean of individual values of *U* for all trees in sample plots.

Results and discussion

In the study stand, the Lorenz index per sample plots ranges 0.38 – 0.73. In the majority of sample plots, the Lorenz index ranges between 0.50 and 0.70, while only in four sample plots, the index is below 0.50 (*Figure 2*). The above values clearly point to a high structural heterogeneity at the plot level.

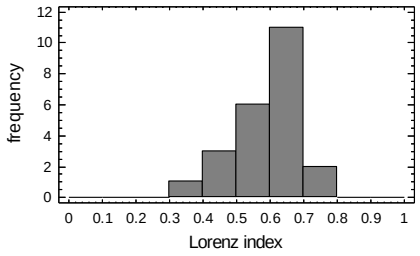

Figure 2. *Distribution of the Lorenz index value by sample plots in the stand 33a*

Lorenz index in the stand is 0.60, standard error 0.02, coefficient of variation 15.40%. The index values reported by Lee et al., (1999) amounted to 0.26 in a relatively homogeneous stand, 0.50 in a clearly structured stand, and 0.58 in a relatively non-homogeneous stand. The Lorenz index points to a high structural heterogeneity in the study stand and at the stand level.

Aggregation index per sample plots was 0.68 – 1.75. On 14 sample plots, aggregation index ranged from 0.80 to 1.20, on 2 plots it was below 0.80, and on 7 plots it was above 1.20. The above values of aggregation index show that the majority of sample plots show random arrangement of tree positions or there is a mild tendency towards a regular tree pattern. In a small number of sample plots, aggregation index shows that se trees are more clumped or have a regular tree pattern.

Aggregation index of the stand amounts to 1.12, standard error 0.05, coefficient of variation 21.69%. At the stand level, the calculated value shows the random arrangement of tree positions with a mild tendency towards a regular tree pattern. Bilek, et al. (2011) established the Aggregation index for the beech stands in the Czech Republic. Virgin stands had values between 0.97 to 1.08, while in managed even-aged stands it went from 1.21 to 1.33.

Aggregation index quantifies rather objectively the spatial structure in the sample plot and stand, but it cannot be applied when a tree position is observed in relation to its neighbours.

This problem can be solved using the indices of individual trees based on the relations of neighbouring trees for the description of the structure on small areas.
Uniform angle index in sample plots is 0.25 – 0.69. In 11 sample plots $\overline{W}$<0.4815, on 3 plots 0.4815<$\overline{W}$<0.5130, and on 9 plots $\overline{W}$>0.5130. Value $\overline{W}$ shows a high heterogeneity of spatial structure per sample plots.
Uniform angle index in the stand is 0.44, standard error 0.03, coefficient of variation 32.17%. The calculated values show that the trees are relatively randomly distributed at the stand level, with a tendency to regular tree pattern.
In the study stand, the mean directional index per sample plots ranges from 0.68 to 2.82. In 17 sample plots $\overline{R}$<1.799, and in 6 sample plots $\overline{R}$>1.799. Just as the uniform angle index, the mean directional index also shows a high heterogeneity of spatial structure per sample plots.
Mean directional index of a stand is 1.55, standard error 0.12, coefficient of variation 36.31%. At the stand level, the calculated value shows the random arrangement of tree positions in space with a tendency to a regular tree pattern.
Diameter differentiation index per sample plots ranges from 0.20 to 0.62. In 7 sample plots $0.0 \le \overline{T} \le 0.3$, in 11 sample plots $0.3 \le \overline{T} \le 0.5$, and in 5 sample plots $0.5 \le \overline{T} \le 0.7$. Diameter differentiation index shows that the differentiation is medium category in the greatest number of sample plots.
Diameter differentiation index of the stand is 0.38, standard error 0.03, coefficient of variation 32.39%. At the stand level, the calculated value shows that the diameter differentiation is medium, i.e. the thinnest-diameter trees in the stand account for 62% of the diameter value of the thickest-diameter trees. Bilek, et al. (2011) found in virgin beech stands in the Czech Republic that diameter differentiation index values were going from 0.76 to 0.78, while in managed even-aged stands it was 0.34 on average.
In the study stand, diameter dominance index per sample plots ranges between 0.25 and 0.86. On 12 sample plots $\overline{U}$<0.5, and on 11 sample plots $\overline{U}$>0.5.
Diameter dominance index of the stand is 0.53, standard error 0.04, coefficient of variation 33.41%. The calculated value shows that, at the stand level, neighbouring trees have a somewhat larger diameter at breast height than the reference trees.
The greatest advantage of the application of modern indices for the quantification of stand structure is that the aspects of stand structure can be expressed numerically as opposed to classical descriptive presentations of structure. Also, a great advantage of structure quantification is that the changes in stand structure can be monitored over a long time period. Since it is established that the beech forests in Serbia are structurally very diversified (Matović, 2012) using these indices can objectively quantify any changes in structure over a longer period of time.
The Lorenz index can be obtained from the data collected for forest stand during the regular forest inventory. The disadvantage is the fact that Lorenz index describes the stand homogeneity, without taking into account the spatial distribution of trees. Aggregation index requires the measurement of positions of all trees on the sample plot. It describes the spatial structure at the plot level. However, it cannot present the changes in spatial structure on a small area (Zenner, Hibbs, 2000). This weakness is especially pronounced in uneven-aged forests where the structure on a small patch is highly variable (Albert, 1999). The indices of individual trees based on the relations of neighbouring trees successfully describe the changes in spatial structure on small patches. These parameters can be assessed at the level of the entire plot when it is necessary to determine the positions of each tree in the sample plot. However, Hui, Albert (2004) proposed the calculation of indices of four reference trees nearest to the sample plot centre. Each reference tree with its four nearest neighbours makes the structural group of 4 neighbours, for which it is necessary to find the positions in relation to the plot centre. Such an approach is called Plus-sampling (Stoyan, Stoyan, 1994) and it

makes it possible to avoid the use of edge correction, which can have a significant impact on the accuracy of index estimation on small plots. Generally, collecting data in the field was simplified due to the modern technologies and tools such as Field-Map technology (IFER-Monitoring and Mapping Solutions Ltd.) and PosTex (HAGLOF).
In common forest practice, e.g. in forest inventory (especially for rapid assessment of spatial structure) uniform angle index, differentiation index and dominance index can be directly calculated in the field without measuring azimuth and distances from the plot centre. Consequently, the above indices are the most simple solutions in practice.

Conclusion

The Lorenz index and diameter differentiation index show that it is the case of a structured stand, both at the plot level and at the stand level. However, the diameter differentiation index shows a somewhat lower value compared to the Lorenz index, which results from the structure which characterises group-selection uneven-aged stands, i.e. such stands are more homogeneous on a small patch than at the plot level or at the stand level. The aggregation index, uniform angle index and mean directional index show random arrangement of tree positions in the stand with a mild tendency towards a regular tree pattern. The diameter dominance index shows that 53 % of the nearest neighbours have larger diameters than the reference tree. The dominance index, in the concrete stand is not a sufficiently indicative measure for the description of spatial structure, however in mixed stands, it is a very good indicator of the relations between different tree species.
All structural changes occurring in the stand as a consequence of management operations and the effects of biotic and abiotic factors can be assessed by the quantification of stand structure using structural indices.

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INTEGRAL EVALUATION OF BELGRADE FOREST RESOURCES FUNCTIONALITY

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Abstract

Until the middle of the past century, forests were treated as natural raw-material resources for wood production intended for mechanical and chemical processing, for civil engineering and thermal energy. Today, we are facing the increasing hazards to the environment and natural ecosystems in general, as the consequence of profit-making, disregarding the ecological consequences. The previous method of "wasting the nature" endangers the human survival and life in general on our Planet. In the past period, especially in the larger and industrial towns in Serbia, forests were not focused sufficient attention to, and there was not sufficient interest of the professional as well as the general social community in the evaluation of forest ecological functions. Taking into account the actual state of the environment of the two-million Belgrade city, and starting from the fact that forest ecosystems are among the most stable terrestrial ecosystems, they should get a much higher significance both in the narrower and in the wider city zone. The common attitude that forest is exclusively for production should be changed, and forest ecosystems near large towns should get new functional priorities in harmony with the principles of sustainable development, protection and enhancement of environmental quality. The paper presents some results of the researching project “Integrated valorization of forest resources in Belgrade” in the light of some of the most significant international regulations of environmental protection, the necessity of biodiversity conservation and sustainable development. The paper also discusses the multifunctional values and significance of urban forests from the aspect of environmental protection and social benefits.

Keywords: *urban forests, functionality, integral evaluation, Belgrade*

Introduction

With intensive development of industry, traffic and other human activity, natural balance on Earth is disturbed. The fact is that we are today faced up with the increasingly excessive endangerment of all elements of the environment and nature ecosystems as a whole, which is the consequence of race with profit, regardless of the ecological consequences. The problems are numerous: environmental degradation and pollution, global melting and climate changes, radiations, water disturbance, impoverishment of biodiversity, etc.

State of environment and significance of forest ecosystems

The sources of pollution in Belgrade and its surroundings are more and more numerous. Increasing amounts of poisonous gases accumulate in the atmosphere, resulting from the combustion of fossil fuels in transport and industry. Agriculture - pesticides and mineral fertilisers are also significant polluters of the environment. Radioactive pollution is a permanent hazard. Demographic growth and many other factors also disturb the ecological balance. The task of modern society is to detect and implement the methods which will not harm the nature. In addition to different technical-technological aspects of environmental protection, the conservation primarily of forest ecosystems as the most powerful and stable systems of maintaining the ecological balance, is very significant. The establishment of forest

cultures and establishment of new spaces of urban greenery of different categories is an irreplaceable form of enhancing the quality of life in general.

In the past period, especially in the larger and industrial towns in Serbia, forests were not focused sufficient attention to, and there was not sufficient interest of the professional as well as the general social community in the evaluation of forest ecological functions. Taking into account the actual state of the environment of the two-million Belgrade city, and starting from the fact that forest ecosystems are among the most stable terrestrial ecosystems, they should get a much higher significance both in the narrower and in the wider city zone (Drazic D., Drazic M., 2001). As the result of the immediate surrounding of concrete, asphalt and metal, concentration of the transport systems and industrial activity within and around the urban areas, the mean temperature values are higher (effect of the "heated island"), the air is drier and polluted, rainfaill are less effectively apsorbed, and noise is significantly higher than in the rural regions.

The aim of paper is to point out that the common attitude that forest is exclusively for production should be changed, and that forest ecosystems near large towns should get new functional priorities in harmony with the principles of sustainable development, protection and enhancement of environmental quality.

Material and Methods

Extensive research in last five years has included the following activities:

Preparatory works (collection of cartographic, literature review, analysis of foreign and domestic experiences in the field of multifunctional evaluation of forest resources in urban spaces)

Field works (vegetation and floristic research, research of vegetation health condition, research of the state of forest resources, research of fauna, soil and floristic sampling) have included the followef forest complexes: Košutnjak with Topčider, Forests and park of Beli and Stari Dvor at Dedinje, Miljakovačka forest, Banjička forest, Zvezdarska forest, Stepin Lug–Baba Velka–Torlak–Jajinci, Makiš and Ada Ciganlija

Laboratory analyses were conducted in the accredited laboratory of the Institute for forestry Belgrade (SRPS ISO/IEC 17025: 2006, LOB 510.01): analysis of soil samples, analysis of heavy metal contents and other hazardous substances in soil and plant samples, determination of agents of phytopathological diseases and insect pests).

The standard soil analyzes and the preparation of samples was applied, by the following methods:

Table1. Applied laboratory methods for the analysis of soil

Analysis	Method
Active and substitutional acidity	Electrometrically LUP504.07
Total humus	Method by Tjurin LUP504.04
Total nitrogen (N)	Method by Kjeldlahlu LUP 504.33
Easily accessible phosphorus and potassium	AL method LUP504.03
The content of free carbonate	Volumetric method LUP504.05
The textural composition of soil	Sedimentation method LUP504.02
Preparation of samples for the analysis of heavy metals	Destruction in nitric acid and H_2O_2

Cabinet processing of field data, documents and results of laboratory analyses (state and classification of Belgrade urban forest resources – age, structure, productivity, degree of conservation, results of comparative research of individual autochthonous species and exotic species development, health condition of forest resources),

Evaluation of the actual functionality (protection, functions of forests and forest soils, conservation of biodiversity and gene pool, base for generative and vegetative reproduction, site-habitat of different flora and fauna species, protection of springs and watercourses, socio-

cultural function, aesthetic function, picnic-recreation function, production function, other functions).
In the aim of assessment of relative value of the main functions of urban state forest in Belgrade administrative region, the modified classifications by Papanek and Vlatković has been applied.

Results and Discussion

Main data on Belgrade forest resources

Total forest area in Belgrade region (with suburban municipalities) is 35.980 ha, of which state forests 20.064 ha and private forests 15.823 ha. Percentage of forest cover in Belgrade region is 11.2% and forest area per capita 0.025 ha. In the municipalities, forest area per capita is very different and ranges from 0.0 ha at Savski Venac to 0.23 ha in Barajevo and Sopot.

Photo 1. Mountain Kosmaj with monastery in the vicinity of Belgrade

Photo 2. Quercetum frainetto cerris Rud. on mt. Kosmaj

As the "minimal optimal value" of forests per a citizen is about 0.33 ha, it is obvious that Belgrade has insufficient land under forest. The situation is moderate by existing different category of urban open green spaces.

Table 2. Open green urban spaces in Belgrade

Category	Hectares
Parks and squares	393
Greenery of the residental parts	1.078
Greenery of the trafficroads	175
Banks, riparian areas, islands	226
Protection zones	36
Greenery of the special categories	350
Total:	**2.258**
Trees in the tree lines	67.063 pieces

The research included the following urban forests in the city of Belgrade administrative region:

Table 3. Objects of research

Locality	Area (ha)
Košutnjak with Topčider	262.71
Forests and park of Beli and Stari Dvor at Dedinje	100.00
Miljakovačka forest	155.95

Banjička forest	41.59
Zvezdarska forest	129.00
Stepin Lug–Baba Velka–Torlak–Jajinci	491.72
Makiš	380.22
Ada Ciganlija	92.65
Forests along motor-way Belgrade–Zagreb to Surčin	159.74
Total:	**1.813.58**

Natural boundaries of Belgrade occur at the borders of two large and different natural entities with three basic biomes: (1) steppe and forest-steppe, (2) broadleaf forests of floodplain and lowland type, and (3) forests with Hungarian oak and Turkey oak. These natural landscapes have been modified by human impact, so that today the prevailing landscapes are urban, suburban and cultivated landscapes.

Forest ecosystems are the most developed form of living-being organisation and the greatest treasury of biodiversity. For this reason, special attention is devoted to the research of forest resources. Major forest types of Belgrade region are represented by the complexes: alluvial-hydrophilous forest types, xero-thermophilous Hungarian oak-Turkey oak forest types, xero-mesophilous sessile oak, Turkey oak and hornbeam forest types and mesophilous beech and beech-conifer forest types. In Belgrade region there are several forest ecosystems: forest of Hungarian oak and Turkey oak, forest of pedunculate oak, forest of silver lime and oaks, forest of pedunculate oak, forest of poplars and willows, forest of sessile oak and hornbeam, forest of pedunculate oak and hornbeam, montane forest of beech and forest of sessile oak. In addition to forest ecosystems, there are also steppe ecosystems of Pontic-South Siberian region and forest-steppes on loess plateaux and hills along the Danube.

Thirty-five tree species have been identified in the region, of which 22 are autochthonous. The most represented species are Turkey oak 18.00%, pedunculate oak 15.40% and Hungarian oak 5.32%. Other species of hard broadleaves have individual percentage of 5%. The most represented soft broadleaf is poplar clone I-214. The percentage of willow, white and black poplar, as autochthonous species, is less than 3.50%. Grey poplar occurs individually at some localities. There are also a great number of alochtonous species which are very well adapted to the existing environmental conditions and achieved very good growth, vitality and decorativeness in adequate locations. To mention a few: *Cedrus atlantica* Man., *Quercus rubra* Du Roi non L., *Liriodendron tulipifera* L., *Taxodium distichum* L. etc. (Drazic D., 1998).

Functions of Belgrade urban forests and other open green spaces

It is necessary to specify some of the most important functions of urban forests in general, in order to access the evaluation of urban forests Belgrade. These are:

Air filtering. Trees very effectively absorb gas pollutants, including CO_2, SO_2, NO_x, etc. The dust is deposed on the trees. It was determined that the tree belt about 30 m wide contains almost the whole dust from air; *Keller* (1979) proved the reduction of Pb for almost 85% behind the protective zone of the trees; Trees also neutralize and almost completely absorb unpleasant smell, replacing them by much more pleasant ones; Helps the increase of the relative air humidity.

Modification of the temperature extremes. Trees, bushes and other vegetation help the regulation of the temperature extremes (Chen, A. et al., 2014); The shade of a big tree can reduce the temperature of the building as 15 air-conditioners (4.220 кJ) on the similar building which is not protected by shade; Energy which is saved by tree planting around the building accounts for 10-50% for cooling and 2-22% for heating (NAA/ISA, 1991).

Noise reduction. Excessive levels of the noise of the majority of the big cities contribute towards the physical and psychological damages. Trees also help by absorbing the noise which is produced by the intensive traffic circulation and numerous activities.

Use of water, re-use and preservation. In the system of parks and forests, stabilisation pools for the treatment of the waste waters can be integrated. Protection of the suburban and rural areas which serve as the water wells for cities must be integrated in the urban plans.
Land protection. Trees and forests are the means of land protection, they protect the sensitive ecocystems on steep inclinations with the sparse vegetation and abundant season rains, and in this way protect human life and their homes.
Biodiversity of the green areas has a vital role for the urban biodiversity.
Green belts and green corridors can serve as the biological corridors, and the connection of the green areas in the network ensured the biological preservation and biodiversity.

Photo 3. Lake on Tresnja surrounded with forests

Photo 4. Recreative zone of Ada Ciganlija with lake in the central zone of Belgrade

Social benefits. Improvement of the esthetic quality of the urban areas - refers to the esthetic and recreation values of the trees, forests and parks; Well-selected and planted trees shelter inevitable visions and provides the privacy, at the same time giving the free view on the remaining part of the landscape; Trees fulfils numerous psychological, social and cultural needs of the population (*Dweyer et al.*, 1991). It has a very important role in release of tension and improvement of the psychological health; The improvement of air quality has a great influence on health, mainly on the decrease of the respiratory diseases.
Health. Urban forests, parks and other green areas provide possibilities for the health physical activities and recreation; The benefits for the physical and mental health of the urban landscapes with trees are proved in the industrialized countries (*Ulrich*, 1984). The studies showed that the patients in the hospitals with a view of the trees recover faster and need the shorter health treatment (*Ulrich,* 1990). Urban forests contribute towards the safety and quality of food.
Employment. Reforestation, landscape arrangement and care of the different categories of the green areas, and particularly agroforest systems, intensify the employment, which is important for the poor cities.
Education and scientific research. In the urban forests, botanical gardens, zoological gardens and other facilities of nature the information centers should be established. They also serve for the scientific research.
Increase of the economic force of the cities and values of the estates. The values of the apartments and houses increase if they are located in the vicinity of trees, for instance by 5% in Hong Kong (*Webb*, 1998), in Finland (*Tyrvainen*, 1999) and even 18% in the USA (*Morales, Micha and Weber*, 1983). In Singapur and Kuala Lumpur the urban area rich in the

forests and trees is an important attraction for the new business and investors (*Kuchelmeister*, 1991).

Urban forests and other categories of the urban greenery unite the people on the work for the welfare of the community and improvement of the environmental quality at the local and global levels (NUFU, 1998).

Integral evaluation of Belgrade forest resources

Table 4. Assessment of relative values of the main functions of urban forests in the Belgrade city administrative region

PRODUCTION FUNCTION				**%**	**PROTECTION-REGULATION FUNCTION**			**%**	**SOCIO-CULTURAL FUNCITION**			**%**	**100%**
Wood Production	Production of safe food and medicinal plants	Game rearing and* hunting*	Production of other products (forest seed, peat exploitation etc.)		Soil Protection	Water Protection	City Micro-climate and air quality		Recreation	Nature Protection and space management	Education		
10	3	-	2	**15**	10	10	10	**30**	30	15	10	**55**	100
-	-	-	5	**5**	10	10	20	**40**	30	20	5	**55**	100
10	2	-	2	**14**	10	10	20	**40**	28	13	5	**46**	100
5	3	-	5	**13**	10	10	16	**36**	15	30	6	**51**	100
15	5	-	-	**20**	20	10	20	**50**	5	20	5	**30**	100
10	5	-	5	**20**	20	10	15	**45**	20	10	5	**35**	100
5	-	-	-	**5**	10	10	25	**45**	25	20	5	**50**	100
5	-	-	5	**10**	5	5	30	**40**	15	25	10	**50**	100
5	3	-	5	**13**	10	20	17	**47**	20	15	5	**40**	100
5	3	-	2	**10**	10	15	10	**35**	40	10	5	**55**	100
10	-	-	-	**10**	5	5	50	**60**	10	15	5	**30**	100

Note: Main forest functions expressed as a percentage (%).

*The production function “Game rearing and hunting“ in Belgrade urban forests does not anticipate. Game rearing and other fauna is presented through the socio-cultural function “nature protection and spatial management“, and it was treated also by the proposal of photo-safari, “Game park“, etc. at the suitable sites.

Conclusion

Taking into account the actual state of the environment condition of the Belgrade, and starting from the fact that forest ecosystems are among the most stable terrestrial ecosystems, they should get a much higher significance and the new universal benefical functional priorities in accordance with the principles of the sustainable development, biodiversity protection, improvement and protection of the enviromental quality were determined.

In the aim of biodiversity conservation and multifunctional utilization of urban forests in the region of Belgrade, besides the higher goals that imply implementation of strategies and measures in the fields of environmental protection, protection of nature, forestry, protection of water and the system of city green spaces defined by the Regional Spatial Plan of Belgrade administrative region and Belgrade Master Plan, the following should be realised in the following years:

conservation of natural forest ecosystems in the direct urban surroundings and protection of ecosystem and species components of biodiversity;

protection and improvement of all water ecosystems;

establishment of new forest stands established on the principles of coenotic diversity;

maintenance and establishment of green area within the urban cores including parks, squares,

tree rows and gardens;
protection of landscape diversity;
evaluation of landscape quality (according to European Landscape Convention) by the establishment of a system of green corridors by integrative model;
increase of total forest area and percentage of forest cover.
In addition to the aforementioned higher goals, it is necessary to realize the following individual goals:
integral evaluation of urban forests functions from the aspect of actual state of environment and demands of the citizens;
identification the priority multiple benefit functions of Belgrade forest resources;
for each forest complex, to determine the percentage of each function ranked by priorities;
by the evaluation of their beneficial ecological functions, contribute to the healthier environment;
to define forest management on the principles that guarantee the conservation and enhancement of optimal forest ecosystem values.

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ADAPTIVE MEASURES IN FOREST ECOSYSTEMS VULNERABLE TO CLIMATE CHANGE

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Abstract

The paper presents the concept of sustainable use of renewable natural resources through: adaptive activities focused on maintaining genetic variability and resistance of species to climatic changes; a change of technique and technology of forest planting; protecting natural areas; improve forest fire protection; improvement of forest resilience to insects and diseases; protection against erosion and floods; improved forest regeneration and sylviculture. Additionally, an alternative use of natural resources through inclusion of the eco-centric approach into adaptive measures has been proposed. The idea of applying adaptive measures is to maintain and build connections between habitats that will allow the movement of insertion and resistance to climate change. However, the consequences can be excessively diverting resources that could undermine the effectiveness of its conservation.

Key words: *adaptive measures, forest ecosystems, climatic change, models, Serbia*

Introduction

Previously recorded changes of climatic parameters and examination of various climate change scenarios (global, regional) indicate that the Serbian territory belongs to a group of regions in which climatic changes endangers sustainable use of natural resources and, consequently, threaten the state of the environment. The most serious problem related to adaptation of forest communities to climate change is the rate of the change. The intensity of change of climate parameters is higher than the natural capacity of many species to adapt to newly-created conditions.

The impact of climate change on forest ecosystems in Serbia

Expected maximal change of temperatures in 21st century could be up to 5. 6°C, so Ratknić et al. (2010) designed modeling experiment with five temperature increase scenarios (for 1, 2, 3, 4 and 5 °C).. Based on the designed model and scenarios, it can be concluded that a drastic change in number and structure of forest ecosystems in Serbia is about to occur in a relatively short period. In addition to the above-stated, it is important that a higher level of risk, related to the expected adverse effects, is associated to relict, rare and endangered forest communities and tree species, which make them distinctive. Considered cumulatively, the above-mentioned effects will have a direct impact on the possibility of preservation of biological diversity and viability of appropriate management of these resources.

The total number of forest habitats in Serbia is 210. An increase of temperature by 1° reduces the number of habitats to 198, whereas a 2° increase reduces the number to 192. An increase of temperature by 3° reduces the number of habitats to 159, whereas a 4° increase reduces the number to 131. A 5° temperature increase reduces the number of habitats to 116, which is a decrease of 44.8%. Number of broadleaf deciduous forest habitats in Serbia is 160. An increase of temperature by 5° reduces the number of habitats by 40%. Of the 32 habitats under conifer forests increase in air temperature of 5°C number of habitats will be reduced by 68.7%, and 18 habitats under the mixed deciduous and coniferous forests of 83.3%.

As the two thirds of the Serbian territory are comprised of mountain terrains, there are species the population of which is limited to mountain peaks, where there are no natural corridors for

their migration. Those species will be among those worst hit by climatic changes, since they are already sensitive, on account of a low population and isolation.
A change of climate creates favourable conditions for arrival and acclimatisation of new alien and invasive plant species. Invasive species drive out autochthonous species from their habitats, change the structure and composition of plant communities and reduce the entire richness of species.

Adaptive activities in forestry

Adaptation ought to reduce sensitivity of forest ecosystems towards climatic changes. Despite the fact that forest ecosystems will adapt to climatic changes on their own, bearing in mind the importance of forests for the society, it is necessary to influence the course and dynamics of adaptation in certain fields. Activities related to a process of adaptation of forest ecosystems must be accomplished now, in order to obtain their full effect in future. Researches in forestry must estimate a long-term impact of climatic changes and determine how and in which habitats should be reacted to this threat. A sustainable forest management must include built-in elements of forest ecosystem adaptation, where it should be emphasized that the inclusion of adaptation to climate change, as a part of sustainable management of forest ecosystems, does not require large financial investments into an unknown future. However, the applied adaptation measures must not have an adverse effect on the present state of forest ecosystems, therefore, an effective adaptation policy must provide a response to a broad spectrum of economic, political and ecological circumstances (Spittlehouse et al., 2003). Adaptation requires:
establishing objectives for future forests, which will be under the influence of climatic changes
raising awareness of the importance of adaptation to climatic changes
determining a vulnerability level of forest ecosystems and forest communities
development of present and future cost-effective flexible activities
forest management aimed at mitigating vulnerability and improving conditions for rehabilitation
monitoring the state of changes and identification of a critical threshold for survival
a successful impact reduction, shortening of a rehabilitation period and reduction of vulnerability towards future climatic changes.
The change of climatic characteristics will cause a shift of species areals towards higher altitudes, along with a shift towards the north. Therefore, it is necessary to guide adaptive activities in afforestation towards maintaining genetic variability and resistance of species with respect to adaptation to these changes, as well as towards changing techniques and technology of planting:
determine the adaptability of species and genotypes in relevant climatic conditions, the boundaries of their transferability, and the development of climate-related characteristics of seed in the zones which are to be changed in the course of time. Provenances should be tested at the boundaries of their ecological range, with understanding of relevant physiological processes (Tyree, 2003).
planting of specific genotypes resistant to pests and showing a higher tolerance to climatic extremes.
planting of forest fruit trees. With the change of climatic conditions, certain forest fruit trees will disappear from some forest ecosystems. As they represent the basis for a regular functioning of ecosystems, genotypes resistant to changes ought to be discovered (Ratknic, 2005).
in selection of species and planting type, forest stands of mixed provenances ought to be established.
change the principle of preservation and re-introduction of rare and endangered plant species.

Rare and endangered plant species usually have specialised ecological needs and low genetic variability (Hansen et al., 2001). A long-term preservation of certain rare species is easier to accomplish in archives (Ratknić, 2005), artificially established stands or arboreta, than in natural stands (Parker et al., 2000).

develop an afforestation technique and technology that will enable survival of seedlings in first years of life in newly-created conditions (mulching, use of polymers, etc) (Ratknić et al., 2010).

By means of adaptation to climatic changes, spreading of genotypes or species adapted to new climatic conditions ought to be enabled. Spreading of non-commercial trees and vegetation will present a particular economic problem. Adaptive activities in the field of forest regeneration involve:

identification of genotypes tolerant to drought (Ratknić, 2007)

aiding migration of commercial tree species from their present to future habitats by means of artificial regeneration (Parker et al. 2000). Shift of species towards the north or higher altitudes may be hindered by inadequate soil conditions, such as lack of nutrient matter, the depth, deficiency of mycorrhizal fungi, etc.

plantation of provenance growing in a broader spectrum of conditions as compared to current habitat provenances

control of undesired plant species, which become competitive species in conditions of changed climate (Parker et al. 2000)

In near future, climatic changes may increase productivity of forests (Cohen, Miller, 2001). However, in long-term, the availability of nutrient matter and adaptation of tree species to a higher content of carbon dioxide may limit productivity. In warmer habitats, stabilisation of productivity at the present level can be expected. Changes may also be expected in forest distribution border areas in the zone of scrub and grass habitats. Based on these changes in forest ecosystems, a change of positions and procedures in the field of forest sylviculture is essential. Adaptive activities aimed at responding to changes are the following:

non-commercial thinning or selective removal of individually driven out, damaged or trees of poor quality, with a view to increasing light, water regime and nutrient matter, which would all be more accessible to remaining trees (Papadopol, 2000). This adaptive measure, however, if applied on larger areas may have an impact on a current regular functioning of ecosystem.

reducing endangerment, with a view to decreasing disturbances in future, by controlling a stand thickness, type and structure of a forest (for instance, supplemental planting, planting of species from subsequent successive phases, etc) (Ratknić et al., 2010).

planting of other species or genotypes, in cases which natural regeneration is not acceptable from the perspective of forest ecosystems in future.

decrease of rotation period and plantation of adaptive species with a view to a more rapid establishment of resistant forest ecosystems.

Rapid changes of forest age structure and changes of terrains can be caused by more frequent and intensive disturbances such as fire, stormy winds and outbreak of pests. There is a high possibility of occurrence of interaction between climatic changes (warmer and drier climate), pest outbreak and forest fires (Fleming et al., 2002).

Adaptive activities must be concerned with changes in the regime of forest fires, which involve:

focusing on protection of regions of high economic and social value (Ratknic et al., 2009).

changing a forest structure (distance between trees, stand thickness, removal of dry upright trees, removal of dead trees) aimed at decreasing a risk and a degree of ecosystem disturbance (Dale et al., 2001). In the process of reduction and removal of dry trees, it should be borne in mind that this material has a particular ecological importance for functioning and preservation of ecosystems, therefore it is necessary to reconcile a threat of fire with the ecological

component (Ratknić, 2011).
development of a ‘smart’ fire landscape. By means of cut, regeneration, stand activities as systems of management of the amount of combustible material in the process of control of fire occurrence and spreading. For instance, an aspen stand, a species resistant to fire, can be planted in several localities in coniferous cultures or in natural forests, with the aim of mitigating vulnerability of ecosystems in large fires. This is an example of an adaptive activity, a positive effect of which will be felt for several decades (Spittlehouse et al., 2003).
increase of a rate of recovery of forest ecosystems after fires (Ratknić, 2011).
Climate change will increase warmth and humidity in present forest ecosystems, create conditions for increased occurrence of insects and diseases. Adaptive activities in protection of forests against insects and diseases may involve:
partial cut or thinning aimed at increasing stability and lowering sensitivity to attacks (Gottschalk, 1995).
sanitation cut of infected trees, where it should bear in mind that this practice may increase the sensitivity of ecosystems to other pests.
shortening of a rotation period with a view to decreasing the period of vulnerability of a stand to harmful insects and diseases (Gottschalk, 1995), aimed at effecting a more rapid transition to more suitable species (Lindner et al., 2000).
use of insecticides and fungicides in situations where other activities are ineffective of inappropriate (Parker et al., 2000).
use of genotypes grown for the purpose of increasing resistance to pests.
Biological and climatic changes will also have implications on use of forests and forest ecosystems. An increased amount of precipitation during winter and a decrease in the summer period may have an impact on water resources in forest ecosystems, increase the risk from erosive processes and endanger fish habitats and hatcheries. Adaptive activities in the field of use of forest ecosystem resources are the following:
an increased amount of wood from sanitation cuts, which are carried out after fires and damage of stands inflicted by insects, along with a reduction of treatment in natural stands left to natural adaptation.
maintenance and rehabilitation of roads, damaged as a result of erosive processes due to an increased amount of precipitation and abrupt snow melt.
mitigation of the impact upon infrastructure, fishery and reserves of potable water.
inclusion of adaptive planning measures into forest certification, as a part of the risk management strategy.
mitigation of climatic changes through carbon management (Ratknić, 2008).
an increased use of biomass as an energy generating product.
development of policy aimed at facilitating creation and implementation of an adaptive response to climate change (Parker et al., 2000).

In order to reduce the drawbacks of the existing global climatic projections, it is essential, for the purpose of quantification of reliability and uncertainty of forecasts, to use regional climatic and impact models.

Conclusions

For forest ecosystems to adapt to climatic changes, it is necessary to preserve forests in their most natural form, with avoiding mono-cultures together with promotion of mixed forests, with respect to both type and age. Maintaining natural or close-to-nature regeneration is recommended, as the method of maintaining genetic diversity and, consequently, reducing sensitivity of forest ecosystems. It is also essential to strategically increase the size and number of protected areas, particularly in exceptionally valuable habitats, create artificial reserves or arboreta, with the aim of preserving rare species and providing multiple protection

to climatic refugia, and to include climatic parameters into the models of forest growth and yield. In artificially established forests and mixed cultures, natural genetic diversity should be fostered, the structure of surrounding forests should be imitated and a direct substitution of a natural ecosystem should be avoided. In order to increase the potential of an ecosystem to respond to climatic changes, it is essential to reduce stress factors that are not related to climatic conditions. With a view to preventing adverse changes in distribution of plant and animal communities, it is necessary to maintain seed sources (seed banks and seed facilities) and increase genetic diversity of trees that are used for establishment of plantation. With regard to seedling nursery production, seedling nurseries should be prepared for potential extension of their capacities, while intensifying experimentation with various plant species and production, growth and plantation of genotypes resistant to drought and other climatic extremes, insects and diseases. It is necessary to build-in the results of regional climatic and impact models into activities that enable timely adaptation to climatic changes, or their mitigation (if possible).

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INVESTIGATION OF POTENTAL OF ALPHA - CYPERMETHRIN APPLICATION FOR COMMON OAK WOOD PROTECTION AGAINST SCOLITID AMBROSIA BEETLES

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Abstract

Scolytid ambrosia beetles present xylophagous group of insects which decrease economic and aesthetical value and technical properties of wood. Damages are highly expressed within high quality veneer wood trunks of Common oak (*Quercus robur* L.) where even minimal damage decreases price significantly. Most threatened are new harvested logs on forest storages which protection is necessary from March till November. In previous decades this problem was solved with application of Lindane (Hexachlorocyclohexane HCH) insecticide, but contemporary regulations excluded its use. Therefore, experiments with use of alpha-cypermethrin insecticide for protection of oak timber was performed.

On June 28th 2012, four experiments were established at Forest estate Sremska Mitrovica of Public Enterprise „Vojvodinašume“ Serbia on two spatially divided localities MU Blata-Malovanci and MU Neprečava Varoš Lazarica. On each locality experiments were divided to Fastac® Forst application with 1% concentration for 1 month period of protection and 2% for 3 month protection period. From each experiment, 4 logs were used for treatment and 4 logs for control. Pesticide consumption was 4 liters per cubic meter of wood. Treatment was performed with backpack blower and assessment was performed 1 and 3 months after application . Efficacy was calculated by Abott′s formula.

Fastac® Forst application in concentration of 1% for protection period of 30 days showed 87.3% and 94.7% efficacy, while application of 2% concentration for 90 days protection period showed 61.6% and 90.23% efficacy. Obtained results indicate good efficacy of application of 1% concentration of Fastac® Forst for oak wood protection for 30 days period.

Key words: scolytid ambrosia beetles, insecticide, protection

Introduction

Scolytid ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) represent very important technical pests of felled logs of oak wood oak at the forest storages. In addition to harvested wood they attack weak oak trees under process of drying, often makes holes in places where trees were mechanical injured. Scolytid ambrosia beetles mostly present secondary even tertiary pests (Mihajlović, 2008). By the construction of the hall in the wood reduces the technical, aesthetic and economic value of oak wood. According to Weidner (1953) technical value of the wood can be lowered by 50 – 90%, while Mirić and Petrović (2005) claim that high quality logs attacked by scolytid ambrosia beetles can decrease its class for 2 ranks what causes lowering of price of timber. Economical value loss of the wood is especially high on damages made on most valuable veneer logs which can be no longer used for veneer production. Scolytid damages can be alleviated by felling of trees during winter and transporting them out of the forest before vegetation started, but in praxis such situations are rare. Also demand for wood in most situations is in collision with working conditions in forest. As alternative a sinking trunks in to the water or permanent water spraying exists, but

those protection measurement are expensive and not to practical in forest conditions. For many decades protection of oak logs were carried out with insecticides based on HCH, but it was prohibited in Serbia in year 2006. After that protection of oak logs became a problem due to the lack of registered insecticides for that purpose. Meanwhile damages and losses were rapidly increased on trunk stocks in forest.

Purpose of this paper was to determine possibility of application of insecticide Fastac® Forst for protection of oak logs on forest log storages and to start registration process for that purpose in Serbia. Significant help during research we had by FE Sremska Mitrovica (PE Vojvodinašume).

Materials and methods

All experiments for protection of english oak logs against Scolytid ambrosia beetles were established on 28.06.2012. at a temporary forest timber stock in FE Sremska Mitrovica (PE Vojvodinašume). In total four experiments were established at Forest estate Sremska Mitrovica on two spatially divided localities MU Blata-Malovanci and MU Neprečava Varoš Lazarica. On each locality experiments were divided to Fastac® Forst application with 1% concentration for 1 month period of protection and 2% for 3 month protection period. From each experiment, 4 logs were used for treatment and 4 logs for control. Insecticide Fastac® Forst content active ingredient alpha-cypermethrin in quantity of 15 ± 2,25 g/l produced by BASF Agro. In all experiments treatment and contol consissts of 4 logs (repetition). Logs in control were not treated. Pesticide consumption was 4 liters per cubic meter of wood, and treatment was performed by backpack blower. Assessment of experiments with 1% concentracion applied insecticide was performed 27.07.2012. Assessment of experiments with 2% concentracion of applied insecticide was performed 09.10.2012. by bark peeling and counting of entrance holes on bark. Efficacy was calculated by Abott′s formula (Abbott, 1925).

Results and discussion

Results of the efficacy of Fastac® Forst for 30-day protection period are presented in tables 1 and 2.

Table 1. Results of the investigation of the efficacy of Fastac® Forst in 1% concentration for log protection from scolytid ambrosia beetles in experiment established in MU Blata-Malovanci

Tretments	Total number of entrance holes	Average number of entrance holes per m^2 of bark	Efficacy (%)
Fastac® Forst	14	1.36	87.3
Control	138	10.72	-

Table 2. Results of the investigation of the efficacy of Fastac® Forst in 1% concentration for log protection from scolytid ambrosia beetles in experiment established in MU Neprečava – Varoš – Lazarica

Tretments	Total number of entrance holes	Average number of entrance holes per m^2 of bark	Efficacy (%)
Fastac® Forst	3	0.3	94.7
Control	59	5.70	-

Results of experiments with 1% concentration showed good efficacy of 87.3% and 94.7% respectively after one month period. Experiments with 2% concentration after 3 months showed only 61.6% in experiment located at Blata-Malovanci (Table 3), while experiment at

second locality (Table 4) showed high efficacy of 90.2%. Obtained results indicate decreased reliability of long period protection suggests 1% concentration with one month protection period. Further research should investigate efficacy of application of 2% concentration for 60-day period what could decrease costs of protection if results show positive results.

Table 3. Results of the investigation of the efficacy of Fastac® Forst in 2% concentration for log protection from scolytid ambrosia beetles in experiment established in MU Blata-Malovanci

Tretments	Total number of entrance holes	Average number of entrance holes per m^2 of bark	Efficacy (%)
Fastac® Forst	23	2.25	61.6
Control	98	5.86	-

Table 4. Results of the investigation of the efficacy of Fastac® Forst in 2% concentration for log protection from scolytid ambrosia beetles in experiment established in MU Neprečava – Varoš – Lazarica

Tretments	Total number of entrance holes	Average number of entrance holes per m^2 of bark	Efficacy (%)
Fastac® Forst	25	2.13	90.2
Control	210	21.8	-

During assessment of experiments more frequent damages of logs were recorded in areas with mosses what probably presented barrier for pesticide. In order to increase protection, mosses should be removed prior to pesticide application.

Due to bark roughness, logs should be treated individually right after the felling in order to evenly distribute pesticide to entire surface of logs. In wood industry, logs stored for longer period can also be damaged by scolytid ambrosia beetles (Maksimović, 1959) and therefore should be also treated with pesticides in order to protect them from insects.

Investigations of efficacy of Fastac® Forst enabled registration and commercialization of this pesticide for common oak logs protection against scolytid ambrosia beetles in the Republic of Serbia. Problem related with use of this pesticide is that active matter alpha – cypermethrin is listed on FSC certificate list of forbidden pesticides (Anonymous, 2015) and therefore Public Enterprise “Vojvodinašume” applied for its derogation of restriction and the process is still ongoing. Derogation permission will enable use of this pesticide for oak logs protection.

Conclusions

Conducted research of investigation of possibility of protection of common oak logs against Scolytid ambrosia beetles showed following:

Experiments showed that application of Fastac® Forst in concentration of 1% with amount of 4 liters per m^3 presents good protection of common oak logs for 30-day period.

Application of 2% concentration can not secure adequate protection of logs for 3 month period of protection.

During vegetation, treatment of oak logs must be conducted immediately after felling and should be continued throughout entire vegetation period.

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IMPACT OF FUNGUS DAEDALEOPSIS CONFRAGOSA (Bolt.: Fr.) J. Schröt. MYCELIUM ON THE LOSS OF WILD CHERRY (Prunus avium L.) WOOD MASS

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Abstract

The paper presents the results of the impact of *Daedaleopsis confragosa* (Bolt.: Fr.) J. Schröt. mycelium on the loss of wood mass in wild cherry (*Prunus avium* L.). This phenomenon was examined on the wooden material (dimensions 4 × 15 × 25 mm) that is taken 20 cm above and below tree breast height, separately from sapwood and heartwood. The fungus *Trametes versicolor* (L.) Lloyd was used as control fungus. The observations were performed on 20 samples in three replications, for each fungus and period of incubation (15 days, 1, 2 and 3 months), separately for sapwood and heartwood. Loss of wood mass was determined as the difference in absolutely dry mass of the sample before and after the fungal treatmant. Statisticaly significant loss of sapwood and heartwood mass was observed immediately after the beginning of the experiment, in the samples infected by *D. confragosa*. In the case of *T. versicolor*, statisticaly significant loss of sapwood and heartwood mass was observed two months after beginning of the experiment. The highest impact on the wood mass loss had duration of wood exposure to fungus ($\beta = 0.696$, $p = 0{,}000$), part of the wood ($\beta = -0.419$, $p = 0.000$) and fungal species ($\beta = -0.237$, $p = 0.000$). During this experiment it was demonstrated that fungus *Daedaleopsis confragosa* caused different types of decay in different tree species. White layered rot was obseved on the wood of wild cherry, linden, willow, alder and Norway spruce, while the white mosaic rot was found on the wood of crab apple and hornbeam.

Keywords: decay, fungi, *Daedaleopsis confragosa, Trametes versicolor*, white rot

Introduction

The fact that the fungi can cause wood decay was accepted after the works of Hartig (1874) who discovered that the fungi actually cause destruction of wood tissue instead of being a consequence of its decomposition. Wood decay develops in dead parts of tree and except for fungi that cause root decay, do not directly endanger life of trees. However, indirectly, wood decay leads to tree breaks and throws under the influence of stormy winds and snow, and make substantial economic damages because usually attacks the most valuable part of the tree (heart), and often starts from the base of the tree which destroys the first most valuable log. According to research of Krstić (1950) in the forests of Istočna Boranja in the fifth diameter degree of beech stands percentage of trees infected by rot fungi ranged from 50 to 85% and the loss of wood mass was above 20%.

Epixylic fungi have an irreplaceable role in the carbon cycling on the Earth, returning into the atmosphere every year, billions of tons of carbon. It is believed, according to Lazarev (2005), that if decay process stopped and the process of photosynthesis continued life on the Earth would be terminated in less than 40 years due to the absence of carbon dioxide. In addition, decay fungi have crucial role in getting rid of wood waste in the soil and make it more fertile. Also, they affect natural pruning and can be applied in medicine *(Fomes fomentarius, Piptoporus betulinus, Ganoderma lucidum, Fomitopsis pinicola, Laetiporus sulphureus,*

Schizophyllum commune, *Trametes versicolor* etc.), some are used for human consumption (*Laetiporus sulphureus*, *Flammulina velutipes*, *Pleurotus ostreatus* etc.) and so on.

Causers of white rot, in the later stage of wood decomposition, cause brighter color of wood in comparison to the color of a normal, healthy wood and are more common in deciduous tree species. This decay is primarily result of lignin degradation, although in a later stage of decomposition, cellulose and hemicellulose are decomposed as well. White nod is never completely white, but there are many types, for example white-yellow, pale ocher, black layered, white ring, white freckled, white mosaic and so on.

A difference in the kind (appearance) of rot caused by *Daedaleopsis confragosa* on different tree species was observed during this research. On wild cherry, linden, willow, alder and spruce it caused layered white rot while on the wild apple and hornbeam caused white mosaic rot. Causers of dark rot in an advanced stage of wood decomposition make wood darker in comparison to a normal, healthy wood and are more common in coniferous tree species. Rot causing fungi firstly break down cellulose and hemicellulose (degradation of the carbohydrate components) but not the lignin. This is a real destructive rot because the structure of wood is destroyed in the final stage, in some species, the whole heartwood is decomposed so that cavities occur, which can be seen after tree falling (Karadžić, 2010).

Material and methods

The mass loss and chemical changes in wood of wild cherry - *Prunus avium* L. (syn. *Cerasus avium* Moench.) was studied on wood samples (tubes) of 4 x 15 x 25 mm dimension, taken from healthy 48 years old trees (age was determined by counting the growth rings on a tree stump), with chest diameter of 37 cm and height of 19 m originating from the National park "Fruska gora". For the analysis was taken the first chuck with the length of 2.0 m and tubes were made from the part of the chuck at breast height (20 cm above and below), particularly in the sapwood and heartwood.

The mycelium of the fungus *Daedaleopsis confragosa* (Bolt .: Fr.) J. Schroet. used in this work, was isolated by standard methods from the piece of wood near the fruit body of the fungus, which was sampled on wild cherry tree from Crni vrh (coordinates N 44^0 08^I $39{,}70^{II}$, E 21^0 58^I $23{,}60^{II}$), at an altitude of 749 m a. s. l. The isolate was stored in mycotheka of Institute of Lowland Forestry and Environment in Novi Sad. Isolate of fungus *Trametes versicolor* (Fr.) Pil., which was used as a test - fungus, was obtained from mycotheka of Department for forest protection, timber and ornamental plants, the Faculty of Forestry in Belgrade.

For test were used tubes (dimensions 25 x 15 x 5 mm) with proper flow of fibers, smooth surface and free of knots and defects. 40 tubes (20 + 20) constituted a control and 20 tubes were used for each fungus and each intended incubation period (15 days, 1, 2 and 3 months), especially for the hardwood and the sapwood. Three replicates were performed so that the number of samples (test tubes) was sufficient for a statistical analysis (Soskic, B. 1994).

After drying and measuring, all specimens were conditioned by the standard method at approximately 12% moisture. The tubes were exposed to the influence of fungus according to the method proposed by Miric (pers. com., 2010).

The experiment was set up in the way that on the fungus was first layed the ring and then on the ring were slowly layed 4 tubes. Ring was enabled fungus to comes in contact with the wood (specimen) and also represented the isolation between the substrate and wood. For each incubation period 5 petri dishes were used, for the hardwood and the sapwood and for each fungus as well. Petri dishes of the same incubation period were then placed in aquariums and on them was put petri dish without the lid in which was 5% aqueous solution of boric acid. After closing, aquariums were placed in a climate chamber General Electronik Modernlab mode. 560, and in total darkness at 25 ± 2^0C. After expiration of the incubation period, the

tubes were taken out from the petri dishes, cleaned from the surface mycelium and dried in a classic oven at 103 $\pm 1^0$C and then measured.
To obtain the difference in mass of healthy wood and wood that has been exposed to the fungus for 15 days, 1, 2 and 3 months. Mass loss of wild cherry wood under the influence of the tested fungi was determined according to the formula:

$$\mathbf{Gm = \frac{m1-m2}{m1} \times 100}$$

Where is:
Gm - loss of wood weight
m1 - mass of absolutely dry wood before exposure to a fungus (expressed in grams)
m2 - mass of absolutely dry wood after exposure to a fungus (expressed in grams).

Results and discussion

For the fungus *D. sonfragosa*, a statistically significant mass loss of sapwood and heartwood was recorded with increasing time (Figure 1). For *T. versicolor* fungus statistically significant mass loss of sapwood and heartwood was recorded in the second and third month (Figure 2).

Figure 1. Mass loss of sapwood and heartwood under the influence of fungus *Daedaleopsis confragosa*

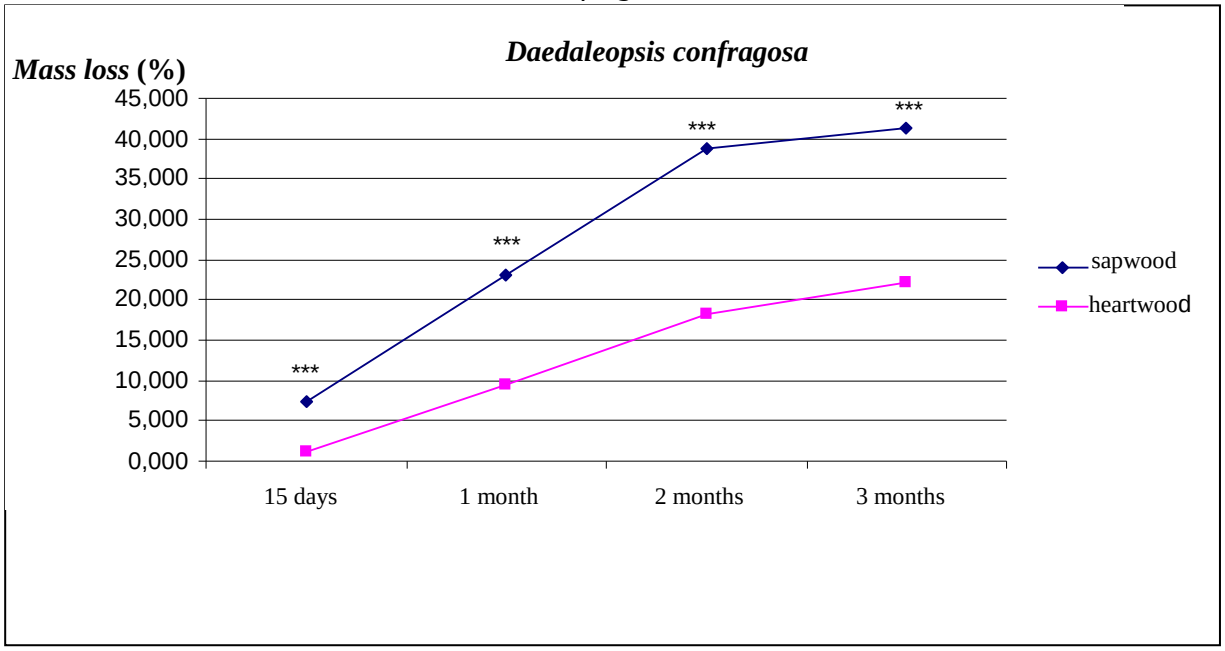
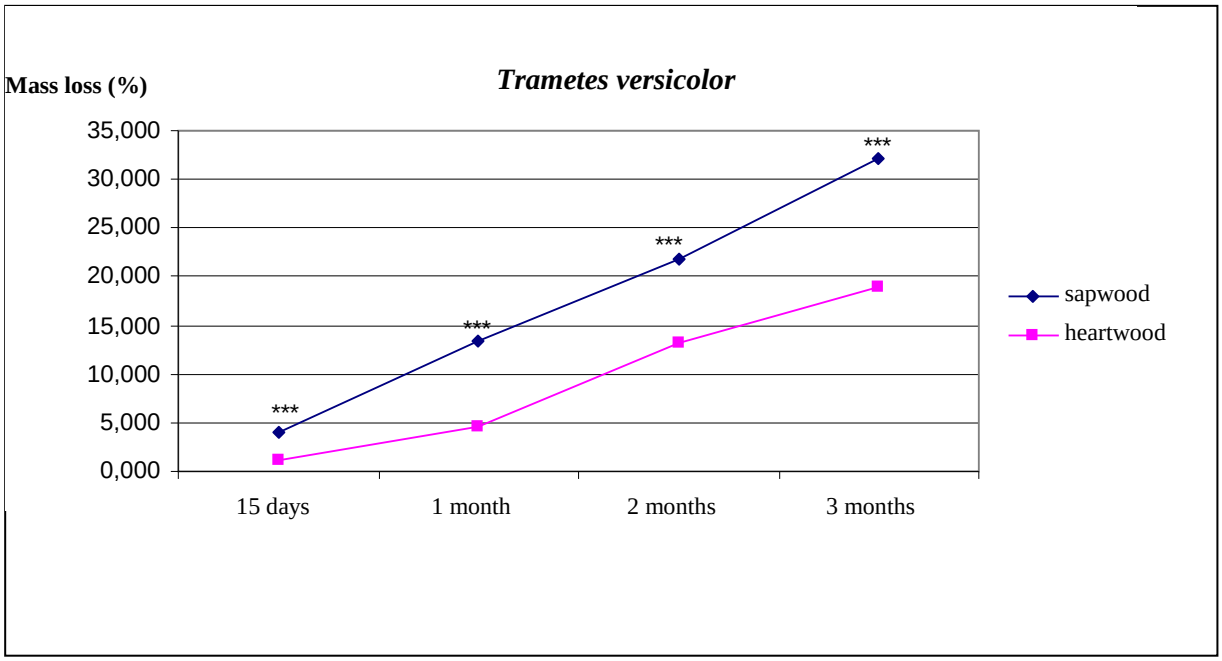


Figure 2. Mass loss of heartwood and sapwood under the influence of fungus *Trametes versicolor*



If the mass loss of sapwood was compared in examined fungi, significantly higher mass loss was caused by *D. sonfragosa* compared to *T. versicolor* in all tested periods (Figure 3.) Although fungus *D. sonfragosa* decomposed more heartwood than *T. versicolor* in all periods, the mass loss was significantly higher only in the first month (Figure 4). The mass loss under the influence of the tested fungi was significantly higher for sapwood in relation to heartwood in all investigated periods.

Figure 3. Mass loss of sapwood under the influence of fungi *Daedaleopsis confragosa* and *Trametes versicolor*

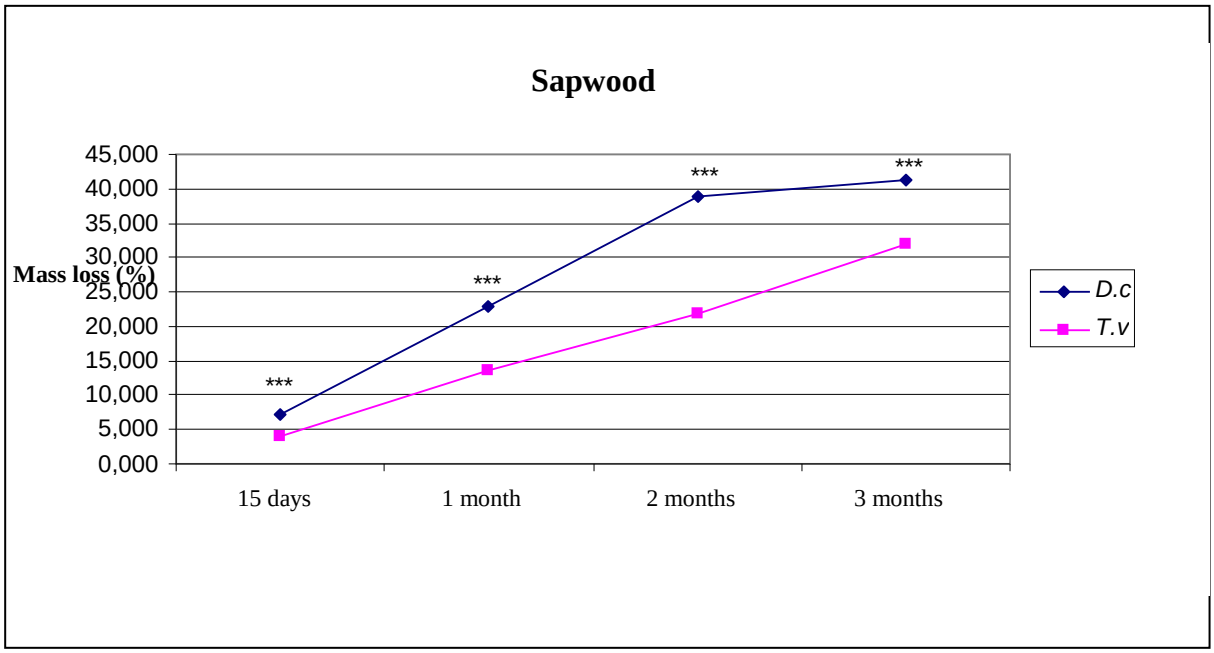
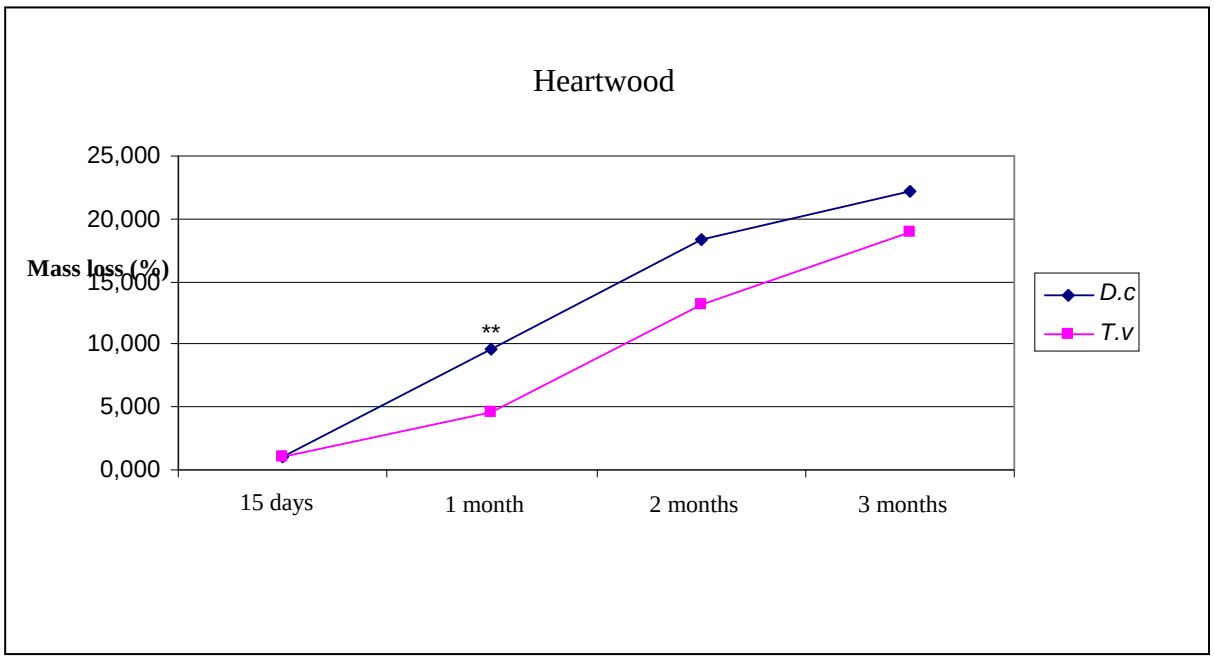


Figure 4. Mass loss of heartwood under the influence of fungi *Daedaleopsis confragosa and Trametes versicolor*



The link between mass loss and joint and partial impact of fungus, type of wood and exposure time to fungus was determined by linear regression analysis. Mass loss is in high correlation ($r = 0.846$, $F = 265.990$, $p = 0.000$) with the joint influence of species of fungus, type of wood and time of exposure (data not shown).

ANOVA analysis showed that mass loss was significantly affected by all studied parameters. The biggest partial influence on the mass loss is the time of exposure to the fungus ($\beta = 0.696$, $p = 0.000$), followed by part of wood ($\beta = -0.419$, $p = 0.000$), and types of fungi ($\beta = -0.237$, $p = 0.000$) (data not shown).

Conclusion

Fungus *D. confragosa*, as a weakness parasite or saprophyte was registered during our studies on wild cherry, alder, willow, linden, hazel, hornbeam, wild apple and spruce. Based on investigation of the effect of *D. confragosa* mycelium on the mass loss of wild cherry wood can be concluded that the tested fungus decomposed much more sapwood and heartwood compared to the control fungus (*T. versicolor*). For the fungus *D. sonfragosa*, with increasing time, a statistically significant mass loss of sapwood and heartwood was recorded. For *T. versicolor* was observed statistically significant mass loss in sapwood and heartwood but only after two and three months. Mass loss was significantly affected by all investigated parameters. The biggest partial influence on the mass loss had the time of exposure to the fungus, followed by part of wood and species of fungi.

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SEASONAL VARIABILITY OF HEAVY METALS LEVEL IN UNDERGROUND WATER OF HYDROMORPHIC SOILS

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Abstract

The paper shows the variation of heavy metals level in underground water in the middle Danube basin. Underground water was analyzed in following hydromorphic soils: fluvisol, humofluvisol, humogley and eugley. Level of following heavy metals: arsenic, cobalt, chromium, copper, iron, manganese, nickel, lead and zinc was determined for a period of two years in three seasons: spring, summer and autumn. Maximum allowable concentration was applied from two sources: Official Gazette of RS 23/1994 and Food and Agriculture Organization (FAO) classification. The results showed levels of manganese above the maximum allowable concentration in all four soil types in all studied seasons, and increased level of arsenic in groundwater of humofluvisol in the summer of 2006, and eugley during the summer of 2007 and copper in groundwater of eugley in summer and autumn of 2006 and in spring in 2007. The level of manganese was below the maximum allowable concentration in certain seasons, in the piezometers which were closer to the stream of the Danube. According to the pollution index (C_d) of the area, heavy metals content varied depending on the underground water level. Thus, at a lower level of underground water, contamination by heavy metals was lower.

Key words: *Maximum allowable concentration, pollution, season*

Introduction

Quality of precipitation water, as well as the presence of harmful substances including heavy metals, gives insight into the overall pollution in a region, which is important for a variety of industries as well as for planning and implementation of environmental policy at national and international level (Špirić et al., 2009).

In order to protect the groundwater is necessary to know the spatial distribution of pollution and the impact of anthropogenic factors (Zahn and Grim 1993). Anthropogenic impact on groundwater quality is primarily influenced by agricultural activity, industry, waste water, settlements and waste disposal (Vlahović, 2004). The consequences of anthropogenic influence are manifested mainly by increased content of nitrates, phenols, total fat, mineral oil and heavy metals which represent a growing problem.

Among all the chemical pollutants, heavy metals are considered as substances with special ecological, biological and clinical significance (Kadović and Knežević 2002, 2004). Presence of one or more of these elements can be significantly increased under the influence of anthropogenic factors at site and remote transport of pollutants. Their concentrations may exceed the critical or toxic levels for the ecosystem or ecosystem component (Kadović et al., 2005). The presence of a compound in a certain amount, might not cause a disturbance in the plant production on one soil type, while on the other type might reduce quality and quantity of yield. If the adsorption capacity of soil or sediments becomes exhausted, the concentration of metal ions in water increases. The accumulation of heavy metals is mostly related to soil clastic, especially to the clay fraction of sediments (Appello and Postma 1994). Heavy metals

reduce the intensity of photosynthesis. As a result the production of plant organic matter is reduced. They also affect the water regime of plants, reducing absorption of water and its transport into the aboveground organs, increase the diffusion resistance of stoma and thereby reduce transpiration (Borišev, 2010). For a more extensive study of hydropedological properties in the Middle Danube region, it was important to determine the level of heavy metals in groundwater as a very significant agent of area pollution. Contamination of groundwater by heavy metals adversely affects the quality of soil and consequently the vegetation at site.

Therefore the aim of our study was to determine the influence of season on heavy metals concentration in groundwater of hydromorphic soil from protected area in the middle part of river Danube basin in Vojvodina, Serbia.

Materials and methods

An area in a protected part of the alluvial plain of the Middle Danube basin in Vojvodina, northern province of Serbia was examined in 2006 and 2007. Groundwater in the area of Vojvodina is situated in the Quaternary sediments and the alluvial deposits and diluvial formations, which are fluvial and eolian deposits, gravel, sands, and loess-like sediments. In the alluvial plain of the Danube the groundwater level oscillations are directly related to the water level of Danube, which feeds groundwater depending on the distance of the riverbed and texture of the soil. The research was done on the terrain which altitude varied from 72.50 to 78.20 m.a.s.l.

This research covered the area of about 14000 ha. On the site samples of groundwater were taken from piezometers to determine the content of heavy metals in different soils of hydromorphic order: fluvisol, humofluvisol, humoglej and eugley. Distance of piezometers from the bank of the Danube ranged from 226 m to 4161 m. The study was carried out on the left bank of the Danube, in a protected part of the alluvial plain. Ten samples of groundwater were taken for chemical analysis from these soils in three seasons: spring, summer and fall, throughout the study period in 2006 and 2007. In total, 60 samples of groundwater were taken. The content of heavy metals was determined by standardized atomic absorption spectrometry procedures, using atomic absorption spectrometer - "Varian 600". The limit values of heavy metal content i.e. Maximum Allowable Concentration (MAC) were applied from two sources: the Official Gazette of RS 23/1994 and the classification of FAO (Ayers and Westcot, 1985). For purpose of statistical analysis arithmetic mean was calculated, as a measure of central tendency, and the results of repeated measurements analysis of variance and the least significant difference test (LSD-test) at the risk level of 5%. On the basis of analytical data, the level of heavy metals exceeding the MAC in all types of soil was determined by the index of pollution (C_d) for examined area. The index of pollution (C_d) quantifies the pollution of groundwater with heavy metals, and combines water quality parameters that exceed the MAC (Nakić et al., 2007). According to Nakić et al., (2007), Backman et al. (1998) gave the following formula for calculating the pollution:

$$C_d = \sum_{i=1}^{n} C_{fi}$$

$$C_{fi} = C_{ai} / C_{ni} - 1,$$

where C_{fi} stands for pollution factor for the i-component, C_{ai} for analytical value of the i-component and C_{ni} for MAC value of the i-component.

Results and discussion

For determination of heavy metals level in groundwater in the area where hydromorphic soils are distributed, sampling was conducted in three seasons during 2006 and 2007. It is important to note that in 2006 level of groundwater was high, while in 2007 the groundwater level was low, i.e. in both years the groundwater level was in correlation with the water level of the Danube (Pekeč, 2010).

Table 1. Repeated measures analysis of variance for levels of heavy metals in groundwater of hydromorphic soils

	Soil (A)	Error $_{(A)}$	Time of measurement[1)] (R)	Interaction A x R	Error $_{(A \times R)}$
	Arsenic				
df[2)]	2	7	5	10	35
F - test	3.092		5.844**	3.421**	
	Cobalt				
df	2	7	5	10	35
F – test	0.619		9.566**	1.138	
	Chrome				
df	2	7	2	4	14
F – test	5.293*		3.218	3.719*	
	Cooper				
df	2	7	5	10	35
F – test	3.822		14.731**	1.895	
	Iron				
df	2	7	5	10	35
F – test	4.107		1.998	1.523	
	Manganese				
df	2	7	5	10	35
F – test	0.126		4.218**	0.73	
	Nickel				
df	2	7	5	10	35
F – test	0.524		9.734**	0.45	
	Lead				
df	2	7	4	8	28
F – test	3.873		2.646	2.679*	
	Zinc				
df	2	7	3	6	21
F – test	2.427		14.809**	1.042	

[1)] Times of measurement within a heavy metal when the values for all measurements were zero did not entered the analysis of variance

[2)] df – degree of freedom

Analysis of variance indicates that variations in levels of examined heavy metals were under significant influence of time of measurement, except for iron and lead (Table 1). However, there was no significant statistical difference in the variation of any heavy metal concentrations in the underground water depending on the soil type. Only in the case of arsenic, chromium and lead the effect of interaction: time of measurements x soil was statistically significant, indicating a significant contribution to the difference between soil types in a deviation from the trend formed under the influence of differences in the times of measurements.

Table 2. Mean levels of heavy metals in groundwater of hydromorphic soils - results of Repeated measures analysis of variance and LSD test at the level of risk of 5%.

Time of measurement	Arsenic		Time of measurement	Cobalt		Time of measurement	Cooper	
1sp	0.0101	bc	1sp	0.0032	b	1sp	0.0408	b
1su	0.0183	b	1su	0.0044	a	1su	0.0473	b
1au	0.0063	c	1au	0.0035	ab	1au	0.0638	a
2sp	0.0115	bc	2sp	0.0015	c	2sp	0.0359	b
2su	0.0304	a	2su	0.0034	ab	2su	0.0203	c
2au	0.0110	bc	2au	0.0012	c	2au	0.0201	c
Time of measurement	Manganese		Time of measurement	Nickel		Time of measurement	Zinc	
1sp	0.6946	ab	1sp	0.0154	b	1sp	0.0164	b
1su	0.7922	a	1su	0.0199	b	1su	0.0415	a
1au	0.6369	abc	1au	0.0181	b	1au	0.0102	b
2sp	0.2227	d	2sp	0.0209	b	2sp	0	-
2su	0.3225	cd	2su	0.0286	a	2su	0	-
2au	0.3840	bcd	2au	0.0076	c	2au	0.0367	a

1sp, 1su, 1au – 2006 spring,summer and autumn , 2sp, 2su, 2au – 2007 spring,summer and autumn

Analyzing the mean values of heavy metals concentrations for years and times of measurement (Table 2) a certain tendency could be noticed: in the summer measured values were the highest, in fall the lowest, and in the spring values were mainly in the middle. This phenomenon is obvious for arsenic and zinc in both years, and for cobalt and nickel in 2007. It was only for the concentration of copper in the first year where the reverse sequence of values was found.

Pollution index of groundwater

According to analyzed pollution index (C_d) can be concluded that in the year with higher level of ground water (2006) higher level of heavy metals were found in groundwater, in all tested soils.

Table 3. Pollution index of groundwater

Piezometer	S3	S6	S10	S16	S12	S13	S14	S4	S15	S18
Soil type	Fluvisol			Humofluvisol	Humogley			Eugley		
Pollution index (C_d)	High underground water level in 2006									
Spring	5.06	1.92	-	11.43	0.88	0.76	-	1.41	4.17	0.21
Summer	5.47	8.25	0.47	5.46	0.90	1.60	0.56	4.49	4.30	0.34
Autumn	3.78	5,27	-	3.13	0.77	2.73	1.89	0.68	4.40	0.34
Average	4.77	5,15	0.16	6.67	0.85	1.70	0.82	2.19	4.29	0.30
Pollution index (C_d)	Low underground water level in 2007									
Spring	1.76	0.03	-	3.30	-	**1.69**	-	-	-	0.17
Summer	0.92	0.31	-	0.74	-	-	**0.82**	2.74	2.84	**1.16**
Autumn	**4.50**	1.31	-	**7.00**	-	-	-	-	1.16	-
Average	2.39	0.55	-	3.68	-	0.56	0.27	0.91	1.33	**0.44**

Bold - high value pollution index of groundwater in 2007 compared to 2006

Only in certain periods in year with lower level of groundwater (2007) the index of its pollution by heavy metals in some soils was relatively high: in the fluvisol (S3) and humofluvisol (S16) in the fall, in humogley (S13) in the spring and in humogley (S14) and eugley (S18) in the summer.

Analysis of the variations of heavy metals in groundwater of hydromorphic soils in different seasons during two years brought the next results. During 2006, with high level of

groundwater in the spring, above the MAC was the level of manganese in all tested soils, except for fluvisol (S10) and humogley (S14). In the summer the contents of arsenic in groundwater on humofluvisol (S10) and the content of copper (Cu) in groundwater on eugley (S18) were above the MAC. The content of manganese exceeded the MAC level in the groundwater of all types of soil. During the autumn values for Cu were still above the MAC for groundwater on eugley (S18), a values of Mn concentration were above the MAC in the groundwater of all types of soil, except for site S10 (fluvisol). In 2007, when the level of groundwater was lower, contamination by heavy metals was lower. In the spring there was higher level of Cu then MAC in groundwater at eugley (S18), and the content of manganese was above the MAC in the groundwater of all types of soil. The number of samples with higher content of manganese than the MAC was lower than in 2006. In the summer concentration of arsenic was higher than the MAC in groundwater at eugley (S4 and S18). Also, manganese concentration was above the MAC within a small number of samples in relation to the summer of last year, but present in the groundwater of all types of soil. In the autumn the level of manganese was also higher compared to the MAC, except for groundwater in humogley,..The level of manganese on sites closer to the Danube was usually below the MAC during low groundwater levels.

Conclusion

In this study was explored area of the Middle Danube Region in the Republic of Serbia, which extends in a protected part of the alluvial plain. In the investigated area groundwater composition was studied to determine the content of heavy metals in ground water in the soil of hidromorphic order: fluvisol, humofluvisol, humogley and eugley. It can be noticed that the manganese content in groundwater at all investigated soils during all investigated seasons in both years was greater than the MAC. However, oncentration of arsenic was above the MAC in the summer and fall of 2006 in the groundwater of humofluvisols and 2007 in groundwater of eugley and the concentration of copper in the summer and fall of 2006 and spring 2007 in groundwater eugley. The pollution index (C_d), in the year with higher level of groundwater (2006) was higher than in 2007 for all types of hydromorphic soil, except for a small number of samples of groundwater where increased only in certain periods.

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ELEMENTS OF GROWTH AND CHARACTERISTICS OF THE FIRST THINNING IN THE MIXED CULTURE OF POPLAR AND BLACK LOCUST ON FLUVISOL

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Abstract

Investigations were carried out in a two-storey culture of Euro-American poplar clone Pannonia, and black locust on the sandy form of fluvisol soil in the area of the upper Danube region (Serbia). In the studied culture, which was established with planting space of 5 × 5 m, and inter-row planted black locust, two experimental plots (EP-1 and EP-2), size of 0.12 ha, were established at close distance.

In the 10 years old culture on each experimental plots was found approximately the same number of trees per hectare, poplar 330-340, which are located in the upper storey, and black locust 2760-2800, which are located in the lower storey. On the EP-1 dominant height of poplar trees amounted 18.7 m, while on the EP-2 amounted 22.4 m. The average diameter of poplar trees on the EP-1 amounted 15.5 cm, and on the EP-2 20.4 cm, and the volume was 52 $m^3 \cdot ha^{-1}$ and 104 $m^3 \cdot ha^{-1}$, respectively. Dominant height of black locust trees amounted 13.0-15.2 m, the mean diameter was 5.2-5.8 cm, and wood volume 44-54 $m^3 \cdot ha^{-1}$, respectively.

In order to foster the growth of black locust trees on experimental plots, the selective thinning were carried out, at which 204 (EP-1) and 169 (EP-2) trees for tending per hectare have been selected and their 482 (EP-1) and 829 (EP-2) direct competitors felled. Thinning coefficient (q_d) was 1.08 (EP-2) and 1.39 (EP-1), indicating thinning from above. Thinning volume on the EP-1 was 14.4 $m^3 \cdot ha^{-1}$ and on the EP-2 was 17.7 $m^3 \cdot ha^{-1}$.

Key words: *mixed culture, poplar, black locust, elements of growth, selective thinning*.

Introduction

Mixed forests are presently on the advance as they are widely held to provide many ecosystem functions and services better than pure stands. The higher resistance and resilience of mixed forests to natural and anthropogenic disturbances combined with the higher biodiversity and carbon storage capacity which result in a higher potential for mitigation substantiate this timely research (Bravo-Oviedo et al, 2014). Mixing has mainly ecological and biological advantages, but in a narrower sense it may also have a strong positive effect on yield and economic efficiency (Redei, 2002).

Two basic principles of species interactions in mixed stands are: (1) „complementarity“, in which two dominant species exhibit reduced competition in mixture compared to that in monoculture, thereby utilising resources more efficiently – also called „the competitive production principle“, „competitive reduction“ or „resource partitioning“; (2) „facilitation“, in which one species positively affects the growth od another in mixture (Kelty and Cameron, 1995)

However, all tree species do not naturally form mixed stands and their establishment and maintenance is often not successful and requires high costs.

In Hungary there are the great interest to establish mixed stands of black locust and white poplar because of their fast initial growth rate and a similar rotation age and have mutual advantages. Planting (mixing) schemes have to be chosen in such a way that they increase the compatibility of the two or more species in the stand (Redei et al, 2012).

Mixed natural stands of white and black poplar with admixture of pedunculate oak and narrow-leaved ash, formerly widely represented in the alluvial zone of the Danube River in its middle course, due to changes of hydrological conditions and vegetation succession, and Euro-American poplar plantation production in the previous decades-long period, today there are only in fragments (Herpka, 1979).

In the former wetland habitats forestry practices in Serbia in the previous work more or less successfully, was established mixed stands of poplar and other tree species in minor areas. However, adequate scientific research that documented and verified the importance of such work, and tending measures in these stands, are left out.

The aim of this study is to highlights the elements of growth in a mixed, two-storey culture of Euro-American poplar clone Pannonia, with planting space of 5 × 5 m, and the inter-row planted black locust on the fluvisol soil and the characteristics of the first thinning in black locust.

Material and methods

Investigations were carried out in a mixed culture of Euro-American poplar (*Populus* × *euramericana* Dode Guinier), clone Pannonia (working title M-1) and black locust (*Robinia pseudoacacia* L.), 10 years old, which is located in the upper Danube region of Serbia (MJ „Apatin protected forest“, section 27, subdivision a). Culture is located on alluvial soil type fluvisol, sandy form, with very variable characteristics in a small area (Živanov 1982). It was established after the clear-cutting of poplar plantations of clone I-214 and complete preparation of the site. So called. „deep“ planting was used for planting seedlings of poplar clone Pannonia at a distance of 5 × 5 m (400 trees·ha^{-1}), while black locust seedlings were planted with so called. „normal“ planting at a distance of 1 m between poplar trees and in inter-row (3600 trees·ha^{-1}).

Two experimental plots (EP-1 and EP-2), with an area of 0.12 ha, a rectangular (aproximately square) shape and a distance of 100 m, were formed in a culture in the autumn of 2014. All poplar and black locust trees on the experimental plots had two cross diameters at breast height measured, with an accuracy of 1 mm. The heights were measured using Vertex III hypsometer on all poplar trees and on a certain number of black locust trees in all diameter degrees, with a width of 5 cm, in order to construct height curves.

In the experimental plots was performed nomination of black locust trees for tending in a number which with poplar trees should be definitive at the end of the production cycle of 25-30 years. Each tree for tending were primarily eliminated main competitors, defined as obstructionist (Klädke, 1990). The intensity of thinning is represented as a percentage of the reduction of the number of trees (N), basal area (G) and volume (V) per hectare before felling (Jovanović, 1988). The numerical definition of the thinning is expressed as the ratio of the quadratic mean diameter of the felled trees to the quadratic mean diameter of the remaining trees, as coefficient q_d (Pretzsch, 2005).

Data analysis included standard procedure for processing the experimental plots in order to obtain the quadratic mean diameters (d_g), the dominant diameters (mean diameter of the 100 thickest trees per hectare, D_{100}), mean height by Lorey (h_L) and dominant (top) height (mean height which corresponds to the diameter of the 100 thickest trees per hectare in the plantation, H_{100}) for each studied tree species. The function of Mihajlov was used for the construction of height curves ($h=a\cdot e^{-b/dbh}+1.3$). Stand volume was obtained using the volume tables for poplar clone Pannonia (Pantić et al, 2013), and for black locust (Cestar and Kovačić, 1982).

Results and disscussion

In the investigated two-storey culture up to 10 years, on the experimental plots, the survival rate of poplar trees was 82-86%, and of black locust trees was 77-78% (Table 1).

Growth elements of the mean and dominant tree of poplar clone Pannonia are smaller in EP-1 compared to EP-2. The difference between the dominant heights of poplar trees on experimental plots of 3.7 m (20%) indicate differences in site index of the stands. Differences in mean Lorey's heigh are even larger and amounts to 4.3 m (25%). The mean and dominant diameter of poplar clone Pannonia in EP-2 are higher by 4.8-4.9 cm (26-32%), i.e. for the entire diameter degree, compared to EP-1. Although there are approximately the same number of poplar trees on both experimental plots, basal area and volume per hectare are lower in EP-1. Basal area per hectare of poplar clone Pannonia in the experimental plot EP-2 are higher by 65% and volume by 99% compared to the EP-1 (Table 1, Figure 1).

Table 1. Basic growth elements in the experimental plots.

Experimental plot	Species	d_g [cm]	D_{100} [cm]	h_L [m]	H_{100} [m]	N [trees·ha^{-1}]	G [m^2·ha^{-1}]	V [m^3·ha^{-1}]
	Poplar	15,5	18,5	17,35	18,72	343	6,50	52,26
	Black locust	5,2	10,3	10,13	13,03	2802	6,03	44,09
EP-1	Total					3145	12,53	96,35
	Poplar	20,4	23,3	21,68	22,41	330	10,74	103,82
	Black locust	5,8	11,4	11,62	15,20	2759	7,21	54,16
EP-2	Total					3089	17,95	157,98

Mean and dominant height of black locust trees on the EP-2 were higher by 1.5-2.2 m (15-17%) compared to the EP-1, while the mean and dominant diameters were higher by 0.6-1.1 cm (11-12%). On both experimental plots was found approximately the same number of 2760-2800 black locust trees per hectare. Basal area of black locust trees on the EP-1 amounted 6.0 m^2·ha^{-1}, and volume 44.1 m^3·ha^{-1}, while those on the EP-2 were greater by 20-23% (Table 1).

The total volume of poplar and black locust trees on the EP-1 amounted 96 m^3·ha^{-1}, and on the EP-2 was 158 m^3·ha^{-1}.

Mean and dominant diameters of black locust trees amounted 0.28-0.34 and 0.49-0.56 of the diameter of poplar trees, respectively. Mean heights of black locust trees amount 0.54-0.58, and dominant heights 0.68-0.70 of poplar tree heights. The relative share of mean and dominant diameters and heights of black locust in relation to the poplar were greater on the EP-1, or on the experimental plot with the lower site index. Similar to heights and diameters, and relative share of basal area and volume per hectare of the black locust in relation to the poplar were greater on the plots with lower site index in which amounted to 0.93 and 0.84, respectively (Table 2).

Table 2. The ratio between growth elements of black locust and poplar trees in the experimental plots.

Experimental plot	Ratio	d_g	D_{100}	h_L	H_{100}	N	G	V
EP-1	Black locust / Poplar	0,34	0,56	0,58	0,70	8,17	0,93	0,84
EP-2	Black locust / Poplar	0,28	0,49	0,54	0,68	8,36	0,67	0,52

Height curves, obtained by the function of Mihailov, show good agreement for both tree species. The coefficient of determination is slightly higher, and the standard error of regression is lower in poplar compared to black locust and indicates a better fit of empirical values with the model (Table 3, Figure 1).

Height curves of black locust have a greater curvature in thin diameters and indicate the presence of trees with smaller diameters and heights. Higher site index (EP-2), causes more pronounced separation of height curves in both, poplar and black locust (Figure 1).

Table 3. The parameters of the height curve model and the elements of the model assessment.

Species	Experimental plot	Model: $h = a \cdot e^{-b/dbh} + 1.3$		Model assessment		
		a	b	R^2	s_e [m]	n
Poplar	EP-1	25.82272	7.587266	0.8651	0.6036	42
	EP-2	28.47813	6.891195	0.8946	0.4581	41
Black locust	EP-1	16.85545	3.933335	0.7563	1.1609	35
	EP-2	19.39680	4.141521	0.8614	1.0832	31

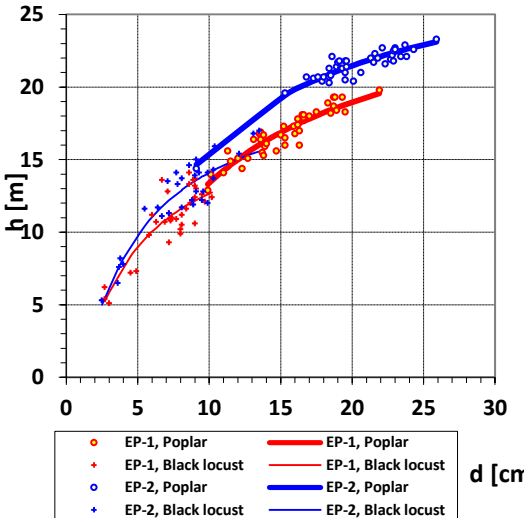

Figure 1. Heigth curves in experimental plots

On the experimental plots nominated 169 (EP-2) and 204 (EP-1) black locust trees for tending per hectare. Mean diameters of black locust trees for tending amounted 8.2-9.6 cm, mean heights 12-14.2 m, and the total volume 8.27-10.16 $m^3 \cdot ha^{-1}$ (Table 4).

Direct competitors of nominated black locust trees for tending were removed in the lower storey, a total of 507 trees per hectare in the EP-1 and 829 trees per hectare in the EP-2. The mean diameters of the felled black locust trees amounted 6.1-6.8 cm and the mean height amounted 11.2-11.5 m. Total of 25 non-perspective poplar trees with a volume of 1.66 $m^3 \cdot ha^{-1}$ were removed on the EP-1. Total basal area and volume, which were removed by the thinning operation, were 1,97-2,39 $m^2 \cdot ha^{-1}$ and 14,43-17,74 $m^3 \cdot ha^{-1}$, respectively (Table 4).

The total number of black locust trees, which were removed by the thinning, was 17% on EP-1 and 30% on EP-2 in relation to a total number of black locust trees on experimental plots. The removed volume amounted 29% (EP-1) and 33% (EP-2) in relation to the total volume of the black locust trees. The thinning coefficient (q_d) was 1.08 in EP-2 and 1.39 in EP-1 and thinning can be described as heavy thinning from above.

Significant variability of the growth elements of trees was found in the mixed two-storey culture of Euro-American poplar clone Pannonia, and black locust on the transect 100 m long, in the coastal part of the Danube, on the soil type fluvisol with very variable characteristics in a small area (Živanov 1982),

The differences of 3.7 m and 4.3 m, which were found between the top and the mean heights of poplar trees, respectively, indicate differences of the one site index class (Marković et al, 1987; Andrašev, 2008). The above is confirmed by the double higher volumes per hectare of poplar on a higher site index (EP-2) compared to a lower site index (EP-1), with a close number of trees, 330-340 trees per hectare.

According to previous research, poplar plantations of Euro-American clones, with spacing of 5 × 5 m, or 400 trees per hectare, provide the greatest amount of wood volume per hectare at a medium and favorable sites (Marković et al, 1997). In the poplar plantations established with a distance of 5 × 5 m, as well as in plantations with a distance of 6 × 6 m, by the age of 10-11 the canopy had been closed by the crowns of trees (Andrašev et al, 2012). In the experimental two-storey culture, despite the high percentage of survival of poplar trees up to 10 years (over

80%), there was no closing canopy on both experimental plots which favored to high percentage of survival of black locust trees on both experimental plots (about 78%) in the lower storey. In addition, black locust trees influenced to the cleaning of the lower branches of poplar trees, and thus to lower costs of tending measures in plantation due to the absence of pruning operation of lower branches that are regularly used in monocultures of poplars.
The growth elements of poplar and black locust trees and the previously mentioned indicates that the mixed cultures of poplar and black locust achieve greater wood volume per hectare compared to the pure cultures (plantations) of poplar at the age of 10 years.

Table 4. The main growth elements of trees for tending and felled trees.

Collective of trees	Experimental plot	Species	d_g [cm]	h_L [m]	N [trees·ha^{-1}]	G [m^2·ha^{-1}]	V [m^3·ha^{-1}]
Trees for tending	EP-1	Black locust	8,2	12,02	204	1,09	8,27
	EP-2	Black locust	9,6	14,24	169	1,21	10,16
Trees for cutting	EP-1	Poplar	11,3	14,21	25	0,24	1,66
		Black locust	6,8	11,22	482	1,73	12,77
		Total			507	1,97	14,43
	EP-2	Poplar			-	-	-
		Black locust	6,1	11,50	829	2,39	17,74
		Total			829	2,39	17,74

On the EP-1, which represents the lower site index class, black locust achieves smaller growth elements of trees and stand in relation to the experimental plot EP-2 on higher site index class. However, the growth elements of black locust trees and stands in relation to the growth elements of poplar is higher in lower site index class in relation to a higher site index class. This could be explained by shading of black locust by poplars, which on a higher site achieve greater heights, diameters and crown, and thus more overshadows black locust in the lower storey. On the lower site index, which represent EP-1, due to the smaller size of poplar trees, black locust trees get larger amount of light, and their dimensions are determined primarily by site characteristics (site class).
On lower site index class trees reach smaller dimensions of diameter, height and crowns volume which causes the higher number of trees per hectare (Assmann, 1970). Given this fact, it can be concluded that maintaining a similar number of black locust trees up to 10 years on experimental plots with different site index classes is caused by favorable lighting conditions in the lower storey. A total number of poplar trees and black locust trees for tending is 499-547 per hectare, which is 25-37% higher than the initial number of poplar trees at the establishment of culture. Direct competitors of nominated black locust trees for tending were removed in the area of crown with the aim of intensifying their growth. Conducted thinning had the character of thinning from above (q_d = 1.08 to 1.39), whereby in the stand at a lower site index class (EP-1) two competitors of each tree for tending of black locust were removed, on average, while in the stand on a higher site index class (EP -2) on average, at least 4 direct competitors were removed.

Conclusion

Based on the number of trees and growth elements of two light-demanding tree species in the two-storey culture, at the age of 10 years, it can be concluded that the black locust trees had enough light for their growth. Growth elements of both tree species are determined by the influence of habitat (site index classes), and the growth of black locust, in great extent, is slowed on the experimental plot with higher site index class. The first thinning was performed

in order to allow more space for growth of selected black locust trees for tending, and heavier intensity is proposed on the experimental plot with higher site index class.

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INJURY OF OZONE – MONITORING PLOT – MOKRA GORA

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Abstract

Induced visible injury by ozone (O3) is that many plant species respond to ambient levels of ozone pollution with distinct visible foliar symptoms that can be diagnosed in the field. The results attributed to LEVEL II Serbia – Mokra Gora sites will be documented in maps covering Europe, characterizing areas of increased ozone risk for European forest ecosystems. Many plant species respond to elevated ambient levels of tropospheric ozone (O3) with distinct specific foliar symptoms. Harmonization of procedures is essential to ensure spatial and temporal data comparability. For the injury data being accepted for the international database and evaluations, National Focal Centers and their scientific partners that are participating in the UN/ECE ICP Forests program must follow the methods and apply the manual described herein. The assessment for visible ozone injury on main tree species is conducted on the leaves from the same branches where foliar analysis is carried out. The samples for foliar injury are collected every second year from the upper sun exposed crown. Methodologies, including quality assurance, such as data harmonization, completeness and plausibility tests have been applied according to the ICP Forests Manual, Parts VIII - Assessment of Ozone Injury. Specific targets are set as follows: quantification of injuries ozone on the selected parcel level II in Europe; detection of temporal trends in the selected plot level II in Europe (significant changes within 10 years with a 95% level of significance of individual plots).

Keywords: *foliar injury, experimental plot, ozone.*

Introduction

The essential basis for choosing ozone (O3) induced visible injury is that many plant species respond to ambient levels of ozone pollution withdistinct visible foliar symptoms that can be diagnosed in the field. The results attributed to LEVEL II sites will be documented in maps covering Europe, characterizing areas of increased ozone risk for European forest ecosystems. However, the development of ozone-induced injury is inter-and intra-species specific, and depends apart from local ambient ozone concentrationson other environmental such asbiotic and climatic factors. Due to the complex nature of the diagnosis and the given restrictions of the investment, results from the tree and vegetation assessment should be considered as semi quantitative.

From July to September, many plant species that are sensitive to elevated ozone concentrations, show visible injuries on the upper surface of the leaf mass. In addition to the apparent symptoms, the leaves of plants damaged ozone is smaller, and the plant may produce a smaller amount of healthy seed. Moreover, injuries may result in depletion of the sensitivity of plants to other damaging agents, such as harmful insects and fungi. In our climate, the best time to observe violations of ozone is from mid-July to mid-September. At higher altitudes, however, a violation of the ozone can be masked by staining the leaves in early fall.

Since the 1980s there are higher concentrations of tropospheric ozone, especially in the warmer half of the year, when the value of the ozone increase depending on weather

conditions and increased anthropogenic activities. While at humans ozone irritates the mucous membranes and restricts lung capacity, in plants attacks and destroys the cell walls or individual cells in the leaves. Over the past 50 years, a large volume of literature has documented O3 impacts on forest trees (see reviews by Kickert and Krupa, 1990; Miller, 1993; Skelly et al., 1997; Chappelka and Samuelson, 1998; McLaughlin and Percy, 1999; Krupa et al., 2000; Bytnerowicz et al., 2003; Percy et al., 2003).

Depending on the sensitivity of plant species and the concentration of ozone, depends and the visibility of damage to leaves or needles. The harmful effects of ozone is difficult to prove, because there is no chemical residues that can be analyzed and measured. Visibility damage on the leaves or needles is the only effect that professionals can easily detect. Tropospheric ozone background concentrations have increased 36% since pre-industrial times (IPCC, 2001). Ozone is known to impact forest trees in many ways including inducing visible foliar symptoms (Chappelka et al., 1999a; Schaub et al., 2005).

The aim of this study was to investigate the long-term, cumulative ozone concentrations in relation to the occurrence and development of visible foliar injury to leaf mass. In addition, in order to assess the impact of environmental factors (abiotic and biotic) that modify the information on air quality in a given forest ecosystems is evaluated experimentally established symptoms in the field, in order to develop a regional risk assessment based on monitoring of ozone and data modeling. So, are evaluated visible ozone depletion in the selected area (Level II) and evaluated the effect of tropospheric ozone at locations where monitors of ozone injury, as well as risk assessment of ozone effects on forest ecosystems. Methodologies, including quality assurance, such as data harmonization, completeness and plausibility tests have been applied according to the ICP Forests Manual, Parts VIII - Assessment of Ozone Injury. Specific targets are set as follows: quantification of injuries ozone on the selected parcel level II in Europe; Detection of temporal trends in the selected plot level II in Europe (significant changes within 10 years with a 95% level of significance of individual plots).

Results from a Stage II will be documented in maps covering Europe, characterized by an area of increased risk of ozone to European forest ecosystems.

Material and methods

The locality on which is a measuring station for monitoring the meteorogical data, within the IPCC project, is located in 20 department, GJ "Mokra Gora - Panjak" in the logging camp "Užice".

Basic features of forest ecosystems on this site are as follows: elevation of 600 m; exposure is northwestern; the locality belongs to the type of white pine forest (*Pinus sylvestris* L.) on clay soil. The culture are 35 years old.

The goal is to collect needles from trees representative of the experimental plot from which the sampling is performed again, twice during the growing season. Sampled needles should then be divided by categories, the one-year and two years.

Score of experimental samples for the presence of damage of ozone is carried out at certain chemical reactions, and the special equipment, by laboratory methodology. For sample preparation is necessary related equipment and a certain amount of dedicated substances. The analytical techniques are used because on the narrow vegetation or tree needles it is difficult to determine damage by ocular methode. In the tables damages are grouped according to the degree of damage and the manner in which recorded occurrence was shown (Tables 2).

The main objective of assessing ozone visible injury on a selected number of Level II plots is to assess the effect of tropospheric ozone at the sites where ozone monitoring is performed, and to contribute to an ozone risk assessment for European forest ecosystems.

For the in-plot assessment, the following variables are measured: Symptomatic leaves or needles, reported as frequency classes (% score of symptomatic leaves for each sampled branch per IM plot).
Ozone visible injury on conifer species is expressed at the upper parts of the crown, in the upper side of branches and needles. For identification follow the criteria below:

- Chlorotic mottling is the most common ozone-induced symptom described for conifer needles; it is the result of chronic exposure to ozone and can be described as yellow or light green areas of similar size without sharp borders between green and yellow zones. However, not all needles in a fascicle may be uniformly affected.
- Chlorotic mottling frequently appears only in needles older than 1 year (second-year needles and older). That is, the observed symptom seems to increase with increasing needle age (age effect).
- Chlorotic mottling is more distinct on light-exposed needle areas in comparison to shaded ones (shade effect).
- It is easier to observe the mottling, if several needles are held close to each other, forming a “plane” of needles.

Results and discussion

Ozone visible injury on conifer species is expressed at the upper parts of the crown, in the upper side of branches and needles. A minimum of 3 branches per tree and 5 trees per plot are assessed. For off-Plot are measured variable, and they are shown in Table 1.

Tabele 1. Scoring and definition for the percentage of symptomatic leaves on a branch with approximately 30 leaves

Score	Frequency class (%)	Definition
0	No injury	None of the leaves are injured
1	1 - 5 %	1 – 5% of the leaves per branch show ozone symptoms
2	6 - 50 %	6 – 50% of the leaves per branch show ozone symptoms
3	51 - 100 %	51 – 100% of the leaves per branch show ozone symptoms

Samples were taken for laboratory analysis from 3 branches for all five trees on which were done evaluation of damage (trees numbered 153, 157, 283, 322 and 350). Also, samples were taken (three branches) from five trees from the edges of the stand. Needles are cut to length by 3 mm and placed in an Eppendorf cuvettes, in which are prepared solution (2.5% glutaraldehyde in Sorrensenovom buffer pH 7.0). Samples are immediately di pinto Eppendorf vials with accurate labels. In lab fixative solution were renew,remove the air in samples by vacum evacuation using an excicator connected to vacuum pump. Then we were done microscopical analysis.
Results are presented in Tables 2 and Table 3. In Table 2 are showen ozone injury of the trees within stands (trees numbered 153, 157, 283, 322 and 350).

Tabela 2. Assessment of damage from ozone on the assimilation organs of ***Pinus sylvestris* L**

No	**153**			**157**			**283**			**322**			**350**		
No of tree	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1															
2															
3															

The results presented in Table 2 show that damages are the same even it depends of possition of the tree in the stands, or from their exposure.
The bole and other crown variables that are associated with growth and overall tree vigor can respond to elevated ozone exposures. Branch mortality in the lowest portion of the crown has been observed in southern California (Parmeter, 1968.) leading to a decrease in vertical crown length, as measured by percent live crown (Stark, 1968.). A reduction in the vertical and radial growth of stems has been documented for ozone-stressed trees in southern California and southern Sierras (McBride, 1975.).



Picture 1, 2and 3 – Level II - Mokra Gora

Conclusion

- Negative operation of photo oxidants with ozone as the main compound was observed on vegetation in Europe since the 80s of the last century. The problems caused by these influences in more detail have been studied over the last decade.
- There is evidence that ambient concentrations of ozone can cause different effects on vegetation including visible lesions on the leaves, reducing growth and yield, and increased sensitivity to biotic and abiotic stresses further. It was concluded that the increase in the concentration of ozone not only has a negative effect on the production of wood (reducing to 10 %), but can also lead to unstable conditions in the forest ecosystem.
- Determining the symptoms of ozone damage to the main tree species in the ICP Forests in bio-indicating points level II subject to ongoing monitoring of the last few years in Europe. Minimum 3 brances by tree and 5 trees by parceles are controled. Experiment were made on red fir and locality Mokra Gora. Based on results, there were no damage by ozone on our experimental plot.

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MORPHOLOGICAL INDICATORS OF QUALITY OF TURKISH HAZEL (Corylus colurna L.) ONE-YEAR-OLD SEEDLINGS

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Abstract

This paper shows the analysis results of morphometric characteristics of Turkish hazel (*Corylus colurna* L.) one-year-old seedlings, their inter-connection and the relation of morphological indicators of the planting material quality that all have been observed in repetitions in three successive years of research. Except the height and the root collar diameter the following morphometric characteristics have been measured: root length, weight of aboveground part of plant (shoot) in absolutely dry condition and weight of underground part of plant (root) in absolutely dry condition. Also, the ratio of diameter and height as well as the quality index of seedlings were calculated. The quality index which includes the height, root collar diameter and their relationship, as well as the weight of aboveground and underground part of the plant in absolutely dry condition and their relationship can be considered the most comprehensive morphological indicator of quality. Beside the quality index the height and the diameter are the good quality indicators, too – especially the diameter due to stronger correlation with the quality index. Both the weight of aboveground and the weight of underground part of the seedlings in absolutely dry condition have the strongest positive dependence with the quality index. The advantage of height and diameter for the assessment of quality is in the method of quantifying these characteristics, because the measurement of the weight is a “destructive” method.

Keywords: *Turkish hazel (Corylus colurna L.), morphometric characteristics of seedlings, quality index*

Introduction

World Wildlife Fund announced in 1999 that the natural biological wealth of the Earth in the period from 1970 to 1995 decreased by 30% (Loh et al., 1999). Therefore, in recent years special attention has been paid to forest fruit trees whose introduction into existing forests and use for afforestation of non-stocked forest land contribute to improvement of the existing status of biodiversity and natural biological wealth.

Turkish hazel (*Corylus colurna* L.) due to its morpho-physiological, anatomical, ecological and technological characteristics has a special place in the forest communities. This fruit tree is a tertiary species which is widespread in the Balkans since the Oligocene (Tucović, 1970). Experience has shown that in afforestation, beside a proper selection of species, the characteristics of the planting material have to be taken into account because that is very important for the success of afforestation. Using of poor-quality planting material can reduce the percentage of acceptance, survival and growth of seedlings in the field and increase the costs for maintenance of forested areas (Oliet et al., 2009).

Determination of planting material quality is mostly based on relatively simple measurement of morphological characteristics (Thompson 1985; Pinto et al 2011) and their good correlation with the success of survival after transplanting (Dey and Parker 1997; Rose et al., 1997). Among the most-used morphological characteristics are the root collar diameter and the height of seedlings. However, these two directly measured characteristics are not individually sufficient to assess the quality (Haase, 2008). Therefore, the derived indicators are commonly

used, such as Dickson's quality index (Dickson, 1960) which connects the height, diameter and weight of seedlings in dry condition; Roller's sturdiness quotient (Roller, 1977), which is the ratio of the height and the root collar diameter.
Calculation of the quality index is an "aggressive" method because it requires to "destroy" a certain number of seedlings for measuring dry mass of shoot and root and to have more measured characteristics. In this research it was separately analyzed the correlation of quality index with directly measured parameters height and diameter.

Material and method

Seed of Turkish hazel was collected from the same tree and planted in three successive years. It was collected from the tree located in the Arboretum of the Forestry Faculty in Belgrade, at 44° 46' 59" N latitude and 20° 25' 29" E longitude, at an elevation of 97 m. Seed was stratified with cold moist stratification (temperature 0-5 °C) in sand for 5 months and thus prepared for spring sowing. 50-100 fruits per meter were sown in early April at a depth of about 5-6 cm. The seed was sown in the substrate *Tref PS fine brown*. The substrate is in the fraction of <8 mm and has a pH of 5.8 (± 0.3) and it is a mixture of peat and perlite in the ratio of 9:1, and peat mixture is 70% white and 30% black peat (Ćirković-Mitrović, 2014).
At the end of the vegetation period on a sample of 90 seedlings were measured height, root collar diameter, root length, oven-dry mass (weight) of aboveground part of plant and of oven-dry weight of root. The weight in absolute dry condition was measured after plant drying in a dryer type *Binder*, at a temperature of 105 °C for a period of 48 hours. It was also determined Roller's sturdiness quotient (Roller, 1977), weight ratio SW/RW and Dickson's quality index (Dickson, 1960).
Basic statistical indicators of morphometric characteristics, as well as the linear correlation between them (Pearson's correlation coefficient) were obtained using software package Statistica 7.

Results and discussion

The average height of measured seedlings in all three years of research is 21.3 cm, ranging from 12.5 to 36.5 cm (amplitude is 24 cm). Most of tested seedlings, 95.6%, were higher than 30 cm. The root collar diameter is 4.58 mm in average, varying from 2.99 to 6.87 mm. The diameter of most plants (43.3%) is between 4 and 5 mm. The average root length is 216 cm; the shortest root length is 107 cm and the longest 420 cm. The average root weight in dry condition is 1.57 g and it is greater than the average weight of aboveground part of seedling, which is 1.43 g. Root weight ranging from 0.75 g to 2.98 g, and the shoot weight ranging from 0.84 to 2.50 g. The ratio of height and root collar diameter is 4.68 and the ratio of weight of aboveground and underground plant part (shoot weight and root weight) in absolutely dry condition is 0.92 in average. The average value of the quality index for all three years of research is 0.54 (Table 1).

Table 1. Basic indicators of descriptive statistics for average values of morphological characteristics of Turkish hazel one-year-old seedlings in three successive years of research

	Mean	Minimum	Maximum	Variance	Std. dev.	Standard error
H (cm)	21.3	12.5	36.5	18.26	4.273	0.260
D (mm)	4.58	2.99	6.87	0.60	0.776	0.047
RL (cm)	216	107	420	4247.14	65.170	3.966
RW (g)	1.57	0.75	2.98	0.14	0.378	0.023
SW (g)	1.43	0.84	2.50	0.13	0.362	0.022
H/D	4.68	2.90	6.83	0.36	0.603	0.037
SW/RW	0.92	0.44	1.64	0.02	0.143	0.009
QI	0.54	0.30	0.90	0.01	0.124	0.008

H – average height, D – diameter, RL – root length, RW – root weight, SW – shoot weight, H/D – height/diameter ratio, SW/RW – shoot weight/root weight ratio, QI – quality index

The statistical analysis of the height of the seedlings showed that there were no significant differences in the size of this morphometric characteristic between three examined years of research (Table 2). The analysis of the root collar diameter of one-year-old seedlings in three years of research showed that in the first and the third year were achieved the significantly greater root collar diameters with respect to the second year (Table 3).

Table 2. Basic indicators of descriptive statistics for the height of Turkish hazel one-year-old seedlings in three successive years of research (2011, 2012 and 2013)

Year	Height (cm)	Minimum	Maximum	Variance	Std. dev.	Standard. error	Coefficient of variation
2011	21.6[a]	12.5	36.5	19.32	4.39	0.46	20.32
2012	21.1[a]	14.1	34.1	21.33	4.62	0.49	21.89
2013	21.4[a]	13.8	30.8	14.44	3.80	0.40	17.76
average	21.3	12.5	36.5	18.26	4.27	0.26	20.05

Mean values in the same column followed by different letter are statistically different for $p<0.05$ (Post hoc Tukey's HSD test)

Table 3. Basic indicators of descriptive statistics for the root collar diameter of Turkish hazel one-year-old seedlings in three successive years of research (2011, 2012 and 2013)

Year	Diameter (mm)	Minimum	Maximum	Variance	Std. dev.	Standard. error	Coefficient of variation
2011	4.84[a]	3.09	6.87	0.72	0.85	0.09	17.56
2012	4.17[b]	2.99	5.38	0.39	0.62	0.07	14.87
2013	4.71[a]	3.16	6.41	0.46	0.68	0.07	14.44
average	4.58	2.99	6.87	0.60	0.78	0.047	17.03

Mean values in the same column followed by different letter are statistically different for $p<0.05$ (Post hoc Tukey's HSD test)

The results of these researches are similar to the results that have been obtained by Ninić-Todorović et al. (2007) that showed that the height of Turkish hazel one-year-old seedlings of different genotypes ranged from 26.77 to 49.27 cm and the root collar diameter ranged from 5.76 to 12.22 mm. However, Ninić-Todorović et al. (2012) in their subsequent researches have found the average height of Turkish hazel seedlings from 13.48 to 17.82 cm, the average root collar diameter of 5.66 to 5.89 mm, the average root weight of 2.93 to 4.18 g and the average shoot weight of 1.80 to 2.81 g depending on the genotype of these seedlings. Considerably higher dimensions in the previous research authors attributed to favorable environmental conditions and intensified tending in the nursery with the aim of forcing Turkish hazel seedlings for successful grafting in the first year of development.

Table 4. Correlation of the studied indicators of quality of Turkish hazel one-year-old seedlings

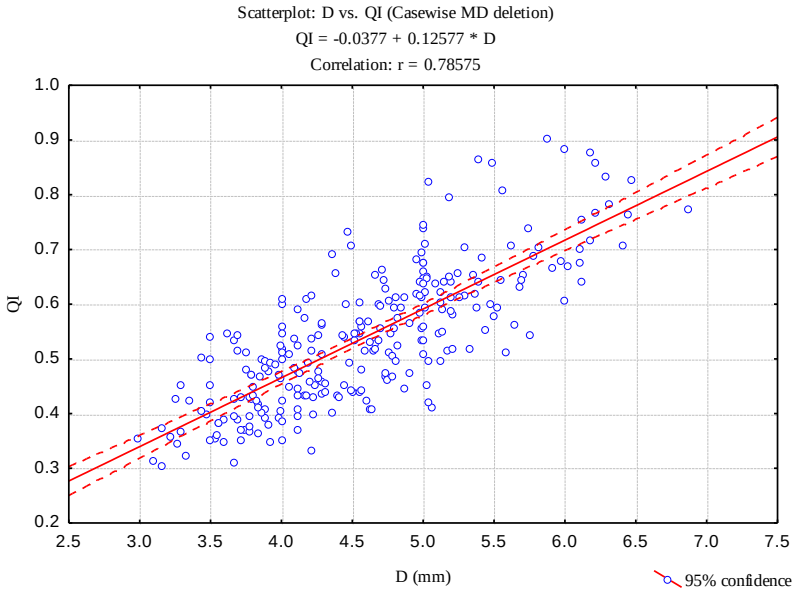
	H (cm)	D (mm)	RL (cm)	RW (g)	SW (g)	H/D	SW/RW	QI
H (cm)	1.00							
D (mm)	0.76**	1.00						
RL (cm)	0.35**	0.47**	1.00					
RW (g)	0.78**	0.67**	0.26**	1.00				
SW (g)	0.68**	0.67**	0.25**	0.81**	1.00			
H/D	0.53**	-0.15*	-0.08	0.31**	0.16*	1.00		
SW/RW	-0.11	0.04	-0.02	-0.24**	0.36**	-0.21**	1.00	
QI	0.54**	0.79**	0.31**	0.85**	0.85**	-0.20**	0.04	1.00

Correlations – Labels for t-test: * - correlation is significant for $p<0.05$; ** - correlation is significant for $p<0.01$ N=180
H – average height, D – diameter, RL – root length, RW – root weight, SW – shoot weight, H/D – height/diameter ratio, SW/RW – shoot weight/root weight ratio, QI – quality index

Between height and all other studied morphological characteristics, except the ratio of weight of aboveground and underground part of the plant in absolutely dry condition, there is a significant positive correlation. The strongest relation is with the weight of underground part in dry condition (0.78) and the weakest with the root length (0.35). A great relationship was recorded between the root collar diameter (0.76) and the weight of aboveground part in dry condition (0.68).

The root collar diameter of seedlings shows the strongest correlation with the quality index (0.79) and the lowest with the root length (0.35). Poor negative significant correlation exists between the root collar diameter and the ratio of height and root collar diameter. A great correlation was found with the weight of aboveground part (0.67) and weight of underground part in absolutely dry condition. Similar relations of morphometric characteristics in one-year-old seedlings of wild cherry determined by Stjepanović and Ivetić (2013) as well as by Binotto et al. (2010) found that the root collar diameter is the best single morphometric indicator of quality, showing a strong correlation with the quality index. The root collar diameter is significant in predicting future performances when transplanting seedlings into the field (Mattsson, 1996; Rawat and Singh, 2000; Davis and Jacobs, 2005) and a method for measuring this characteristic is simple in comparison to measurements of characteristics necessary for calculating the quality index.

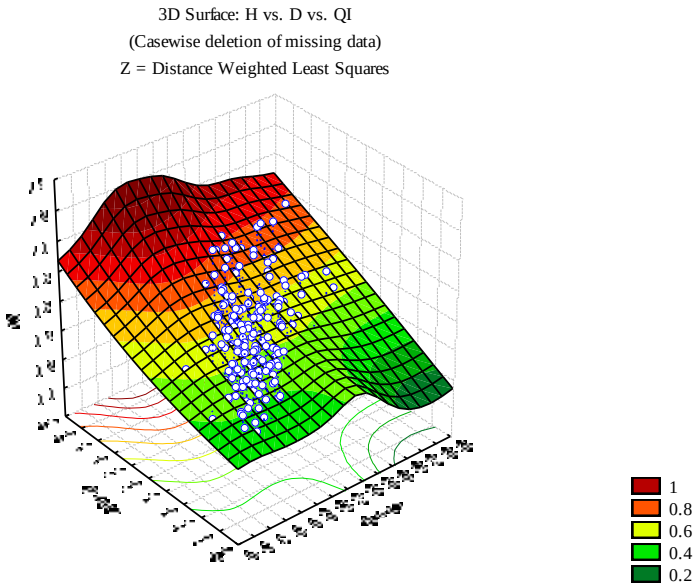
The graph 1 shows the dependence of QI and root collar diameter.



Graph 1. Regression analysis – linear dependence of quality index and root collar diameter

The strongest significant correlation was found between the quality index and the weight of aboveground part (0.85) and underground part of the seedlings (0.85) in dry condition. This was expected due to the method of calculating the quality index (Binotto et al., 2010; Stjepanović and Ivetić, 2013).

Graph 2 shows the dependence of quality index variable in relation to the height and the root collar diameter. With the increasing of the root collar diameter constantly is being increased the value of the quality index. When increasing the height of seedlings with the largest root collar diameter the quality index increases to average value of the height and then it starts to decrease. In seedlings with smaller root collar diameter the value of the quality index is equable. In seedlings whose height is around 20 cm to seedling with height of around 26 cm the quality index increases and afterwards its values decline.



Graph 2. *Area of a combination of height (H) and diameter (D) as a predictor as opposed to Quality Index (QI) as the dependent variable*

Conclusions

The analysis of morphological indicators of quality of Turkish hazel one-year-old seedlings led us to the conclusion that the height and the root collar diameter are good quality indicators, but the root collar diameter shows stronger correlation with the quality index than the height.

Both the weight of aboveground and the weight of underground part of the seedlings in absolutely dry condition have the strongest positive dependence with the quality index. The advantage of height and diameter for the assessment of quality is in the method of quantifying these characteristics, because the measurement of the weight is a “destructive” method.

The quality index which includes the height, the root collar diameter and their relationship, as well as the weight of aboveground and underground part of the plant in absolutely dry condition and their relationship can be considered the most comprehensive morphological indicator of quality.

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APPLICATION OF GENETIC MARKERS IN DETERMINATION OF FOREST TREES SEED ZONES IN SERBIA

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Abstract

Studying genetic potential and characteristics of different habitats of seed stands of black pine (*Pinus nigra* Arnold), Norway spruce (*Picea abies* Karst), Scots pine (*Pinus sylvestris L.*), and beech (*Fagus moesiaca* Domin./Czezccott) was base for separation of forest trees seed zones of these species in Serbia. In conveyed studies the testing of structure of the genome of their population was done by protein markers application as the most often used polimorphic markers at the level of gene products and molecular DNA markers based on PCR phenomenon. Results of analyses revealed that interpopulation diversity within these species was greater than that registered among their seed stands. Genetic distances between analyzed seed stands were obvious, so the future seedlings production as well as domestic and international trade of seed material should be realized in accordance with these results. On the basis of obtained results the seeds zones were separated, and of Austrian pine, spruce, Scots pine and beech in Serbia were defined.

Key words*:* *Austrian pine, spruce, Scots pine, beech, seed zones, Serbia*

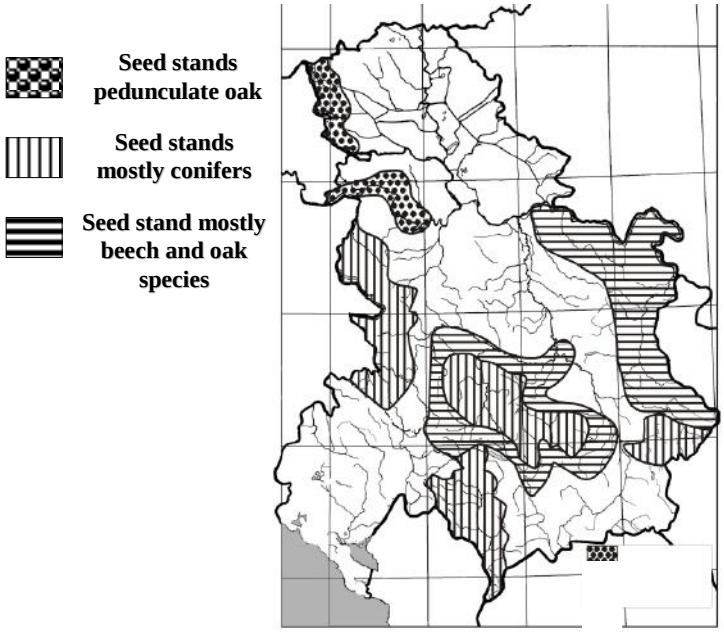
Introduction

Study of potential and spontaneous trait variability, which influences the generative multiplication and yield of tree species in a lesser or a greater extent, is necessary for organization of production and seed material trade control. Production, trade and usage of seed of tree species should exclusively be organized at the level of seed zones, seed stands, and seed trees, Isajev et al. 1999, 2002. Revision of existing, and nomination and separation of new seed stands of economically important species such as Austrian pine (*Pinus nigra* Arnold), Norway spruce (*Picea abies* Karst), Scots pine (*Pinus sylvestris L)* and beech (*Fagus moesiaca* /Domin./Czezccott) are permanent activities, and they are of a primary significance for forestry in Serbia. These activities should be synchronized with activities of lumber camps and scientific institutions because some stands are aged or destroyed by climatic factors or insects, diseases and anthropogenic factors, and they should be replaced with new and young stands satisfying prescribed criteria, which should be registered as seed objects, Djorović et al. 2003. Success of these activities is associated with separation and limitation of areas where appropriate new selections can be used. Aimed at running the above mentioned activities, the investigations encompassed the following:

- Determination of the precise area of these species in Serbia
- Geographic distribution, geographical latitude and longitude, scope of heights above sea level, area, location;
- Definition of ecological characteristics of their populations – climatic, pedological, phyto-cenological etc.
- Separation of seed zones, and
- Creation of maps of seed zones in GIS

Material and methods

To meet the need for the increasing quantity of diverse and selected forest seeds, numerous seed sources were designated in Serbia, to ensure the production of good quality nursery stock. According to the Register of Sees Sources of the Ministry of Agriculture and Forestry of the Republic of Serbia, the number of seed sources in Serbia is 310, on the area of 2,135.85 ha, which is 0.095% of the total area of the forest land in Serbia (2,360,400 ha)., map 1.



Map no 1. Distribution of natural seed sources in Serbia

Establishment of field experiments network enabled testing of seed stand of Norway spruce, Austria pine, Scots pine and beech, i.e. seed zones of these species in Serbia. Multi-annual studies in Serbia included ecological characteristics and stand condition of seed objects and productivity and yield quality of individual subpopulations, which is the basis for location of seed objects, i.e. separation of seed zones. Studies at population level – in seed stands and in laboratories (Faculty of forestry, Belgrade, Institute of forestry, Belgrade, and in the Laboratory of biotechnology, Institute of maize “Zemun Polje”, Zemun) have been carried out since 2003. Fieldwork was carried out in seed objects, table 1.

The following activities were completed in seed stands, table 1:

- Test trees were separated by applied method of individual selections (candidate, evaluation and marking). Ten test trees were selected as working sample in each seed stand - total of 460 mother trees were separated for analyses.
- Collecting plant material – cones and seeds for laboratory analyses was collected.
- Cones and beach nuts were selected and seeds were extracted for determination of genetic similarity i.e. genetic diversity of these species growing in different populations;
- For determination of genetic similarity i.e. genetic diversity of different populations of Norway spruce, Scots pine and beech under laboratory condition one year old assimilation organs were used;
- Collected plant material was properly packed and frozen until usage.

In the laboratories of Faculty of forestry and Institute of forestry in Belgrade samples were prepared for biochemical and genetic analyses using crypto technique and mechanical processing, aimed at determining the degree of similarity between seed zones within the same and different regions.

Table no 1. Seed objects where field works were carried out

SPECIES	FOREST ESTATES	TOTAL NUMBER OF SEED STAND
Austrian pine *Pinus nigra Arnold,*	„Forestry" Raška (2), L.C. "Prijepolje" Crijepolje, L.C. "Užice" Užice, L.C. "Stolovi" Kraljevo, TB „Goč“ Faculty of forestry, Beograd	**6**
Norway spruce *Picea abies L.*	Prijepolje,. Užice,. Kraljevo, Raška, National park Kopaonik, Kruševac, Pirot and in PE National park Tara	**11**
Scots pine *Pinus sylvestrisL.*	«Golija-Ivanjica, «Rasina»-Kruševac, «Užice» Užice, «Forestry-Raška, «Prijepolje» Prijepolje, P.E. «National park Kopaonik», PE »National park Tara»;	**7**
Beach *Fagus moesica Domin, Maly Czec Zott*	Kruševac, Ivanjica, Kragujevac, TB „Debel i Lug“ in TB „Goč“ Faculty of forestry, Belgrade, Zaječar, Užice, Beograd, N.P. Fruška Gora, N.P. Đerdap, N.P. Kopaonik.	**22**

Determination of genetic similarity i.e. diversity of different seed zones of the above mentioned species was done in the Laboratory of biotechnology of the Institute of maize “Zemun Polje” Zemun, using standard methods for protein isolation (according to Wang C.,et al.1994) from seeds and DNA marker technique based on PCR method (according to Jones C.J. et al.1998,

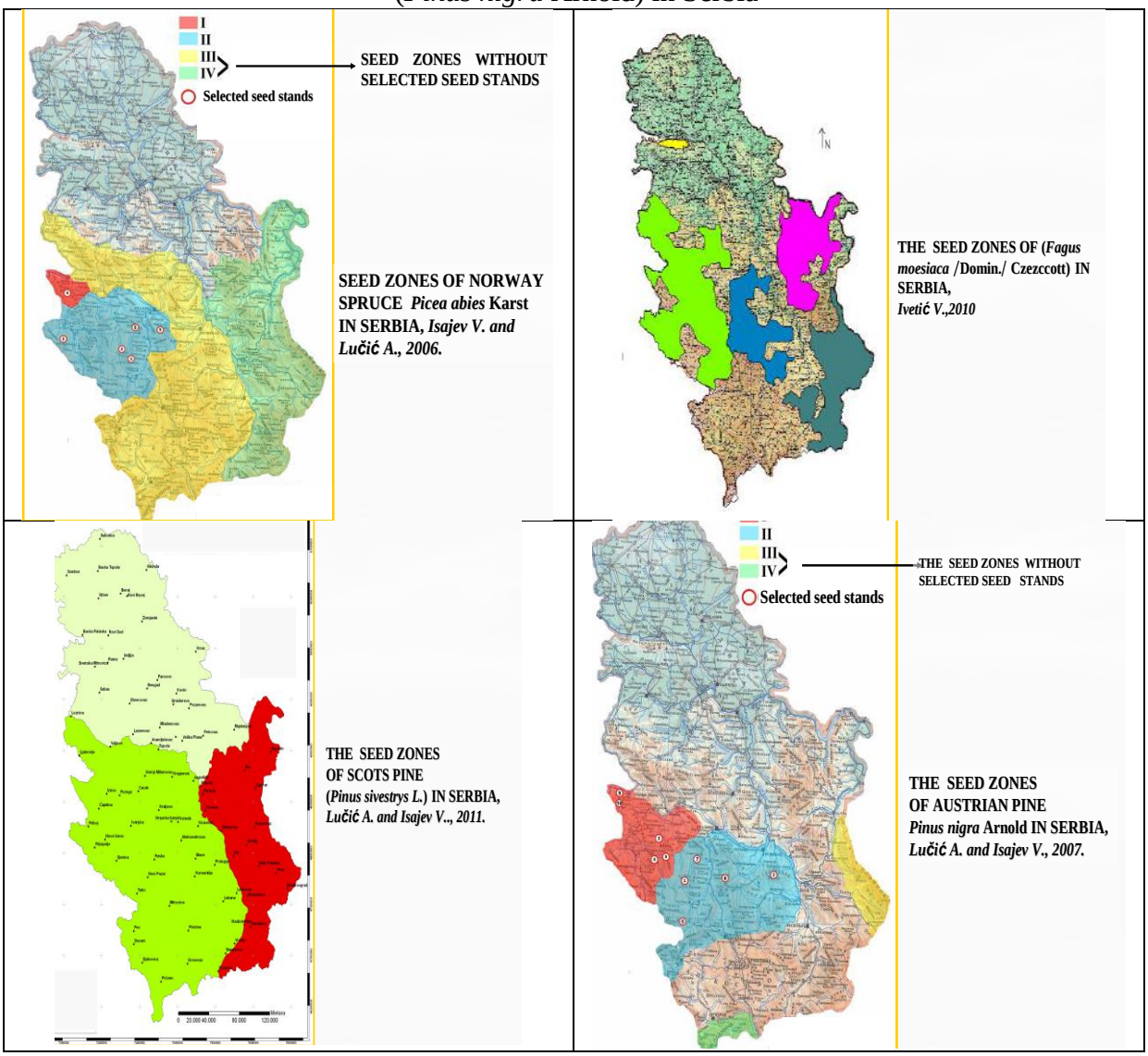
Results and discussion

Spatial distribution of seed zones, map 2. encompassed the greatest part of cenological, ecological and population diversity in Serbia. Improvement of forest seed science, application of contemporary methods of forest genetics and forest typology led to better understanding of the type of adaptability, productivity and quality of individual subpopulations, which provided production of quality seed of economically important tree species. Separation of seed zones was initiated by ecological-geographical studies, and analyses of genetic distance between seed stands run by application of genetic markers made it precise.

Investigation of genetic structure of the seed zones of these species acquiring information regarding group and individual variability. Applied genetic markers were the tools for better understanding the nature of; a) genetic similarity, i.e. diversity of populations studied by analysis of protein complexes from assimilation organs; b) interrelation of ecological traits characteristic for habitats from which samples originated. Results obtained by applied genetic markers revealed that inter-population diversity was higher than it was registered within studied Norway spruce, Austria pine, Scots pine and beech seed stands. On the basis of obtained results seed zones in Serbia were defined in accordance with the Law on reproductive material of forest trees (Articles 6 and 7), map 2.

Since genetic distance was determined between analyzed seed stands intended for nursery production, the trade of seed material should be realized in accordance with defined seed zones regions.

Map no 2. Spatial distribution of seed zones of, Norway spruce (*Picea abies* Karst), beech (*Fagus moesiaca* /Domin./Czezccott) Scots pine (*Pinus sylvestris L)* and Austrian pine (*Pinus nigra* Arnold) in Serbia



The aim of preserving genetic specificity of natural populations of these species has two aspects: genetic differentiation, and preservation of gene pool of these species (*in-situ* and *ex-situ*).

Areas and continuation of Norway spruce, Austria pine, Scots pine and beech forests are such that it is practically impossible to separate greater complexes of seed objects, so the selection must be directed toward separation of group of trees or individual trees, Mataruga et al. 1998, 2005. According to the **Spatial plan of the Republic of Serbia** and **National Strategy of forest development**, by which the huge work relating to aforestations was predicted, it is necessary to separate the seed zones and seed objects in these areas as the carriers of seed and seedlings production.

Analyses of protein content in assimilation organs of 400 test trees were used to test genetic structure of seed stands and some information on population and individual variability of

spruce, Austrian pine, Scots pines and beech was obtained. Interaction between ecology traits characteristic of habitat of population from which assimilation organs originated and the results obtained by analysis of protein complexes was determined.
Analyses results revealed that assimilation organs of different seed stands had different protein patterns. Differences in number, distribution and intensity of protein fractions were determined. Genetic similarity within each seed object was calculated on the basis of analysis of needle-associated protein complex taken from mother trees. Cluster analysis of tested populations revealed expressed genetic diversity of studied populations illustrated by a dendogram.
Obtained results are the basis for future work on development of model of crossing of test trees of different seed stands, and it is supposed that the obtained hybrid seed will show heterosis. On the basis of: ecological characteristics of seed objects, yield quality obtained by biochemical analyses, and determinations of genetics distances among Austrian pine, spruce, Scots pine, and beech seed stands, the maps of seed zones for these species in Serbia were created (Maps 2,). These maps make the basis for more reliable trade of seed material within Serbia, and they are also needed for issuing documentation for the purpose of exportation.

Conclusions

The orientation to desired properties of seeds and nursery stock for the afforestation programmes requires the abandoning of the technology of seed and nursery stock production at the species level, because their quality is difficult to guarantee. Seed production should be organised at the level of seed zones, i.e. seed stands. Studying genetic potential and characteristics of different habitats of seed stands of black pine (*Pinus nigra* Arnold), Norway spruce (*Picea abies* Karst), Scots pine (*Pinus sylvestris L*.), and beech (*Fagus moesiaca* Domin./Czezccott) was base for separation of forest trees seed zones of these species in Serbia, and their transfer into the seed traits.
By studying individual or group variability and the genome structure of these speciess using protein markers as the most often used polymorphic markers at the level of gene products, and molecular DNA markers based on PCR phenomenon we should get better acquainted with the nature:

1. Mutual relationships between ecological traits, which characterize habitats of Austrian pine, Norway spruce, Scots pine, and beech stands, from which analyzed seed and assimilation organs originate, and results obtained by laboratory analyses;
2. Degree of variability of certain morphmetric characteristics of seed, and assimilation organs, and existence of mutual dependence between studied characteristics;
3. Genetic similarity, i.e. diversity among studied populations;
4. Separation of Austrian pine (*Pinus nigra Arnold*), Norway spruce (*Picea abies Karst*), Scots pine (*Pinus sylvestris L*.), and beech (*Fagus moesiaca* /Domin./ Czezccott) seed zones regions in Serbia.
5. Separated seed zones are presented on maps: four for Austrian pine, Norway spruce and Scots pine, and five for beech.

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ANALYSIS OF VARIABILITY AND DEVELOPMENT OF DOUGLAS FIR PROVENANCES IN CENTRAL SERBIA

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Abstract

Douglas-fir is one of the most productive and economically most important conifer species in Canada and North America. The large natural range in horizontal (from British Columbia to New Mexico) and vertical (from sea level to 2800 meters above sea level) terms is the reason of existing the great ecological, population and genetic variability of the species. Introduction of the species with such a wide natural range inevitably requires testing by the provenance test method. The analysis of development, adaptation and progress of plants in new site conditions is an important prerequisite for the selection of promising provenances.

The provenance test of Douglas-fir with the original seed originated from North America was performed in the system of field trials (random block system) on the area of 2.5 ha on the mountain Juhor in the Central Serbia. In this paper are presented results of development of 20 Douglas-fir provenances and these results refer to 28-year period since the founding of the experiment (plants at age of 32 years). The best results in terms of survival, diameter, height and volume growth showed the provenances from lower elevations of the coastal part of federal state Washington.

Keywords: *Douglas-fir, provenances, diameter, height, volume.*

Introduction

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is one of the most productive and economically most significant conifer species in Canada and USA. The large natural range in horizontal (from British Columbia to New Mexico) and vertical (from sea level to 2800 meters above sea level) terms is the reason of existing the great ecological, population and genetic variability of the species and a consequence of that is a great number of provenances (Göhre, 1958.). Introduction of the species with such a wide natural range inevitably requires testing by the provenance test method because it is necessary to have a prior knowledge about thriving of individual provenances.

Institute of Forestry in Belgrade in 1977 got seed of 20 different provenances originating from North America. The aim of this study was to determine which provenances grow best in Serbia, and for that reason three field trials in different environmental conditions have been established. This paper presents the results of field trial in mountain Juhor in central Serbia.

Material and methods

Douglas-fir field trial is located in FMU “Juhor I” department 53d managed by Forest enterprise Juzni Kucaj Despotovac, Forestry administration Jagodina, on the site of mountain beech (*Fagetum moesiaca montanum* Jov. 1976) forests, on elevation of 660-700 m. Douglas-fir seedlings were produced in the nursery of the Institute of Forestry in Belgrade from seed imported from North America. The seed originated from 20 provenances that have a different latitude, longitude and elevation and they cover a part of the natural range of species (Table 1). Produced seedlings were transplanted into the field (in 1982) at the age of 2 + 2 and the field trials were established on the principle of randomized block design, so-called “random

block system”. In the field trials were regularly carried out measures of tending and protection of seedlings.

Table 1: Geographical characteristics of Douglas –fir provenances from North America

Code	Mark	Latitude (° N)	Longitude (°E)	Elevation (m)
Oregon 205–15	1	43.7	123.0	750
Oregon 205–14	2	43.8	122.5	1200
Oregon 202–27	3	45.0	122.4	450
Oregon 205–38	4	45.0	121.0	600
Washington 204–07	9	49.0	119.0	1200
Oregon 205–13	10	43.8	122.5	1050
Oregon 205–18	11	44.2	122.2	600
Oregon 202–22	12	42.5	122.5	1200
Washington 202–17	15	47.6	121.7	600
Oregon 201–10	16	44.5	119.0	1350
Washington 201–06	17	49.0	120.0	750
Oregon 202–19	18	45.3	123.8	300
Oregon 205–11	20	45.0	123.0	150
New Mexico 202–04	22	32.9	105.7	2682
New Mexico 202–10	23	36.0	106.0	2667
Oregon 202–31	24	44.3	118.8	1500
Oregon 205–29	26	42.6	122.8	900
Oregon 205–08	27	42.7	122.5	1050
Oregon 204–04	30	45.0	121.5	900
Washington 205–17	31	47.7	123.0	300

In spring 2010, at plants age of 32 years or 28 years since the founding of the experiment, the measurements of diameter at breast and height were carried out and the survival of seedlings was recorded. Diameter at breast height was measured with an accuracy of 1 mm and height of the seedlings with an accuracy of 1 cm. For studied characteristics were conducted descriptive statistics, analysis of variance and for all testing the error of 5% was considered as statistically significant. Statistical analyses were done using the statistical package Statgraph 5.01.

Results and discussion

The survival of the plants ranged from 25 to 91.7 %. The highest percentage of survival had the provenance Oregon 205-08 and the smallest percentage the provenances Washington 204–07, Oregon 205-18 and Oregon 202-31 (table 2). Better results in terms of survival showed the provenances from lower elevations of western part of the species range. Similar conclusions in their researches reached (Dittmar, Knapp, 1987; Orlić, Perić, 2005; Schultze, Raschka, 2002).

Table 2. Survival of the plants

Code	Mark	Survival %
Oregon 205–15	1	58.3
Oregon 205–14	2	83.3
Oregon 202–27	3	75
Oregon 205–38	4	41.7
Washington 204–07	9	25
Oregon 205–13	10	66.7

Code	Mark	Survival %
Oregon 205–18	11	25
Oregon 202–22	12	50
Washington 202–17	15	66.7
Oregon 201–10	16	41.7
Washington 201–06	17	66.7
Oregon 202–19	18	75
Oregon 205–11	20	66.7
New Mexico 202–04	22	33.3
New Mexico 202–10	23	58.3
Oregon 202–31	24	25
Oregon 205–29	26	50
Oregon 205–08	27	91.7
Oregon 204–04	30	58.3
Washington 205–17	31	66.7

The diameter at breast height of plants at age 32 ranged from 13.8 to 32.1 mm. The best result had the provenance Oregon 205–29 and the worst the provenance Washington 204–07. Maximum value of diameter at breast height of 43.3 cm was measured in provenance Oregon 205–14. Minimum value of diameter at breast height of 12 cm was measured in provenance Oregon 201–10. The greatest distribution of measured values of diameter at breast height around mean value was in provenances which had a more intensive growth (table 3). As with the plant survival, better results in terms of diameter at breast height showed the provenances from lower elevations of western part of the species range.

Table 3. Data on diameter at breast height of the plants

Code	Mark	Mean	Max	Min	SD
Oregon 205–15	1	25.6	33.6	14	9.02
Oregon 205–14	2	26.1	43.3	20.2	6.10
Oregon 202–27	3	24.5	34.5	13	6.70
Oregon 205–38	4	27.7	32.8	18.4	6.52
Washington 204–07	9	13.8	15.6	12.8	1.56
Oregon 205–13	10	26.9	30	22	2.87
Oregon 205–18	11	30.5	34.8	26.8	4.03
Oregon 202–22	12	30	40.7	22	7.01
Washington 202–17	15	23.4	31	13.9	5.74
Oregon 201–10	16	16.8	29.4	12	7.26
Washington 201–06	17	18.3	26	13.3	3.79
Oregon 202–19	18	27	30	23.3	2.16
Oregon 205–11	20	23.6	30	12.9	5.26
New Mexico 202–04	22	23.2	29.3	19	4.36
New Mexico 202–10	23	20.2	27.6	14.5	4.31
Oregon 202–31	24	19.2	25	14.8	5.26
Oregon 205–29	26	32.1	37.4	27.8	3.32
Oregon 205–08	27	23.5	35.3	14.8	6.98
Oregon 204–04	30	23.3	30	16	5.47
Washington 205–17	31	25.5	34.5	18.2	5.60

The height of plants at age 32 ranged from 11.5 to 22.7 m. The best result had the provenance Oregon 205–11 and the worst the provenance Washington 204–07. Height maximum value of 24.7 m was measured in provenance Oregon 205–13. Height minimum value of 11.5 m was measured in provenance Washington 204–07 (table 4). The distribution of measured values of height around mean value was greater in provenances which had a more intensive growth. As with the plant survival and the diameter at breast height, better results in terms of the height of the plants showed the western provenances from lower elevations.

Table 4. Data on height of the plants

Code	Mark	Mean	Max	Min	SD
Oregon 205–15	1	19.4	20.1	18.3	0.95
Oregon 205–14	2	21	21.9	20.3	0.81
Oregon 202–27	3	18.4	20.2	16.3	1.96
Oregon 205–38	4	20.6	21.6	19	1.40
Washington 204–07	9	11.5	10.7	11.5	0.57
Oregon 205–13	10	22.2	24.7	20.9	2.14
Oregon 205–18	11	21.3	23.4	18.8	2.34
Oregon 202–22	12	20.1	24.3	19.1	2.92
Washington 202–17	15	19.1	19.7	18.5	0.60
Oregon 201–10	16	15.4	18.8	11.6	3.61
Washington 201–06	17	19.8	23	15.8	3.67
Oregon 202–19	18	21.9	23.1	20.9	1.12
Oregon 205–11	20	22.7	24.4	20.8	1.81
New Mexico 202–04	22	16.9	18.2	14.6	2.02
New Mexico 202–10	23	15	17.3	11.8	2.84
Oregon 202–31	24	14.6	14.6	13.2	0.99
Oregon 205–29	26	19.7	20.7	19	0.91
Oregon 205–08	27	18	20.6	16.6	2.25
Oregon 204–04	30	18.8	20.5	17.5	1.55
Washington 205–17	31	22	24	20.6	1.75

In provenance tests of Douglas-fir in Austria the best results have shown the provenances from lower elevations so their use is recommended in the future (Schultze, Raschka, 2002). The best provenances in Germany have been those from western part of the peninsula Olypic and western from Caseades Washington (Kleinschmit at all, 1991). The best results at plant age of 10 years in terms of quality and growth intensity in Bosnia has shown provenance Alberni from British Columbia from elevation of 150 m (Pintarić, 1989). In Douglas-fir trials in eastern Germany at plant age of 27 years the best results have shown provenances from lower elevations (Dittmar, Knapp, 1987). In Croatia the best results in terms of plant growth and survival have shown provenances from federal state Washington from lower elevations-to 300 m (Orlić, Perić, 2005). In Serbia at plant age of 20 years better results in terms of plant growth and survival have shown provenances from western part of the species range from lower elevations (Lavadinović et al. 2001).

Conclusion

The results on survival, diameter and height growth of Douglas-fir provenances presented in this paper make easier the operation of afforestation in conditions of Serbia and at the same time they show how significant is the seed origin for introduction in new stand conditions.

Results of growth and adaptability of Douglas-fir provenances at age of 32 show the potential of particular provenances. The best adaptive and development characteristics show provenances at lower elevations from western part of the species range.

Initiated researches have to be continued at later stages of development and it would certainly be very useful to conduct a research on the second generation. Only after these findings we could make more reliable conclusions and give recommendations for future afforestation.

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EFFECT OF FOREST SITE TYPE ON THE GROWING STOCK OF FOREST-FORMING SPECIES UNDER CONDITIONS OF THE DNIEPER STEPPE, UKRAINE

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Abstract

In the article, it was carried out analyze of age structure and detection of regularities in average growing stock within stands from *Pinus sylvestris* L. and *Robinia pseudoacacia* L. under forest site conditions within the Dnepropetrovsk region. *Robinia pseudoacacia* stands reach the maximum growing stock (193.9 m^3/ha) in conditions of high moisture at the age of fifty years. Soil moisture influenced significantly on the timber volume per hectare in stands from *Robinia pseudoacacia*. *Pinus sylvestris* reached the highest values of average growing stock (323 m^3/ha) under mesic conditions in 7^{th} age class. Average growing stock in *Pinus sylvestris* under wet-mesic and wet habitat conditions was three times higher than the one under very dry conditions. Rising of soil fertility determined increasing of average growing stock in the *Pinus sylvestris* stands. The highest values of average growing stock were registered in 8^{th} age class within the bir and subir, and in 7^{th} class within the suhrud. When increasing the age, it is observed the maximum values of growing stock in the 8^{th} and 13^{th} age classes in the *Pinus sylvestris* and *Robinia pseudoacacia* stands, and decreasing of growing stock values at a later time. Under different moisture conditions, contrasting energy of growth of *Pinus sylvestris* and *Robinia pseudoacacia* caused significant differences in development and structure of their stands with age. Due to transition from optimum to the worst soil moisture conditions, it was observed decrease of shoot, fir needle and leaves formation, crown density, size of individual trees, and their resistance.

Key words: *Pinus sylvestris* L., *Robinia pseudoacacia* L., Dnieper Steppe, age structure, growing stock

Introduction

Poor forest cover in Steppe zone of Ukraine does not exceed 3–5 % of the total native area. It determines the spread of negative climate changes, especially desertification and deflation (Medvedev, 2001). In the Steppe zone, it is planned new forests stands with various functional purposes, where *Pinus sylvestris* (Scots pine) is actual species to create forests of recreational and functional categories. *Robinia pseudoacacia* (black locust) is used as species for protective afforestation (Koval et al., 2002).

Large variety of area and landscape within the Steppe zone provides a wide range of habitat conditions and their climatic characteristics. Therefore, to ensure reforestation and afforestation in the Steppe, it is need to consider in detail the types of habitat conditions (Geltman, 1983; Golubets, 2007; Koval et al., 2002; Turkevich et al. 1973; Vedmid′, 2010). Thus, it is important to use the combination of forest typology approach and forestry practice principles being an essential basis for successful functioning of forests in the Steppe. These issues were considered by the authors in scientific publications (Loza and Nazarenko, 2006; Bezrodnova et al., 2013; Lakyda and Lovinska, 2014; Lakyda and Sytnyk, 2014). An organism and environment unity allows considering the plant communities and their habitats as an integral consistent system. Rise of soil moisture and amount of organic matter within the sequence of trophogen gradation causes numerous qualitative transitions and leads to changes

in behavior of physiology-biochemical processes. It affects to productivity changes as an integral indicator of plant growth and development.

Current forest ecosystem survey is devoted to identification of mechanisms for formation of maximally efficient forest stands, and to determine effect of edatope abiotic factors on phytomass accumulation processes. Search of interdependence between abiotic habitat conditions, qualitative and quantitative productivity characteristics of the tree species is necessary for some applied purposes, formulation of forest management directions, and further development. According to E.S. Migunova, typological approach application improves significantly on quality of forest management. Issues of forest stands creation and cleaning cutting must be solved in accordance with the certain forest types (Migunova, 2014). Background of forest typology represents a reliable estimation of the forest site capacity in new forest planting and identification of definition of land typological. It allows to increase greatly the efficiency of forest melioration in natural areas, where lack of soil moisture is the main limiting environmental factor (Migunova, 2014).

To achieve maximal implementations of grow capacity and development of forest-forming species, it is necessary to establish influence pattern of the basic edatope characteristics: soil fertility (trophotope) and moisture (hygrotope) on the values of average timber resource in the main forest-forming species, as a quantitative forestry-biological index. The goal of this survey was to analyze of age structure and to detect of relationship in average timber resource formation for *Pinus sylvestris* and *Robinia pseudoacacia* under forest site conditions (nutrient status and soil moisture).

Materials and methods

The survey was conducted in the forest plantations with Scots pine and black locust subordinated to the Dnepropetrovsk Administration of Forest and Hunting Management during 2009–2014 years. Analysis of age structure and average growing stock in Scots pine and black locust was conducted on the basis of stratum database in forest inventory materials with mathematical statistics (Medvedev, 2001; Vedmid′, 2010). The indexes of actual area and growing stock have been used to calculate age classes that combine in age groups. The total sample size for Scots pine was 5158 stratums with the total area 21472.9 ha, and for black locust it was 4739 stratums with the total area 17683.7 ha.

The classification schemes of trophotops and hygrotops is based on the edaphic factors, such as soil fertility and humidity. Soil fertility is characterized by trophogenic sequence and denoted by the letters A, B, C, and D. Each individual unit of trophogenic sequence called trophotope. Each trophotope is represented by the forest site with equal soil fertility within its boundaries, distinguished from the next by one gradation (Pogrebnyak, 1955). Trophotope "A", "bir" or “pure pine forest”, indicate very poor soil conditions, predominantly with sandy soil, sometimes loamy sands with a short rhizosphere zone; gritty consistency stipulate their poorness. It includes peaty soils, which occurred as a result of swamping by the sphagnum (raised) type. Vegetation is exclusively oligotrophic.

Trophotope "B", "subir" or “pine mixed forest” is characterized by relatively poor soils. It is represented by loamy sand or sandy soil with thin sandy-loam or loamy layers, or with a heavy layers of that at a considerable depth. In the other cases, this trophotope is represented by sandy-loam and loamy soil with low thickness of soil layer, including skeletal soil on mountain slopes. It includes the peat soil with transitional swamping. The vegetation consists of pine-forest oligotrophes with admixed mesotrophes.

Trophotope "C", "suhrud" or “oak mixed forest” has a relatively rich habitat conditions. Soils are represented by sandy-loam, sometimes sand with layers of loam and sandy-loam, or denuded shallow grey forest soil, sometimes brown soil with the little humus horizon, skeletal, derived from volcanic rocks and sandstones. Vegetation is composed of

oligo-, meso- and megatrophes. Trophotope "D", "hrud" or “oak pure forest” has the most fertile habitat conditions. The soils are loam with the heavy (greater than 0.8 m) rhizosphere, more rarely sandy and sandy-loam soils with the layers of loam and clay, available to plant roots. Sometimes the sandy and sandy-loam soils occur with shallow horizon of flow “mineralized” groundwater. This includes also the richest soils of lowland swamps. Megatrophes dominated in composition of climax forest vegetation.

Whereas, the trophotopes are divided by six hygrogene ranges: *hygrotopes* with indicators of soil moisture 0 to 5 (Pogrebnyak, 1955; Sviridenko et al., 2004). Hygrotope of 0 range respond to very dry (xerophilic) conditions; 1 – dry-mesic (meso-xerophilic); 2 – mesic (mesophilic); 3 – wet-mesic (meso-hygrophilic); 4 – wet (hygrophilic); 5 – swamp (ultrahygrophilic).

Very dry types. The habitat conditions include the top of sand dune hills, denuded soil of the beam foreheads, and drained upland edaphotopes within the Chernozem Steppe. Groundwater under these conditions is very deep, and the only source of water is precipitation, which is able to moisten the top of soil, usually dry, only for a short period of time. ***Dry types.*** Conditions for sandy soils with low water storage capacity; dryness of them depends on the groundwater depth; on the clay soils it depends on dryness of climate, surface runoff (on the slopes), high evaporation (southern exposure), and low total water capacity (shallow and lithosolic clay soils of mountain slopes).

Dry-mesic types. Sandy soil in well-moistened habitat is provided by higher groundwater level (2–4 m); in the loamy lands the groundwater is deeper than 4 m, often outside the layer available for plant roots. In southern regions, the satisfactory moistening is carried out by surface runoff reducing, increasing total soil water capacity, and more complete saturation of the soil by winter precipitation (steady winters).

Mesic types. In southern regions, good moisture is provided by the same conditions as in the previous type, and in Northern regions due to the better drainage (tops of hills, slopes). The groundwater level in sandy soils is 1–2 m, and 2–4 m in loamy and clay soils. The subsoil is blowdown, the land form is undulating or gently rolling.

Wet-mesic types. The overwetting habitats. Increasing of soil moisture depends on the higher groundwater level (at a depth of about 1 m in the sands, and 1–3 m in the sandy-loam soil). ***Wet types (bog forest)***. The habitats have excessive moisture and peat soils. Much of vegetative period, the groundwater level is near of the soil surface (Sviridenko at al., 2004).

According to the classification scheme, each site of forest is characterized by two classification units: trophotope and hygrotope. In the surveyed forest stands we selected 20 sampling units for black locust and 16 sampling units for Scots pine in existing grades of trophotopes and hygrotopes within stands of different ages. The sampling units delimitation was carried out instrumentally with surface marking and binding to net of rides. The size of sampling units was ranged from 0.25 to 0.5 hectares. Within the sampling units it was determined the total growing stock with the model trees by way of step representation. Model trees were selected for each level of thickness. Work on the sampling units was performed in the following order:

1) complete tree enumeration;
2) determination of the mean diameter of the stands with usage of average cross-sectional area according to tally sheet, by dividing the sum of basal areas on the total number of trees within the forest stand;
3) selection of three medium-size and shape sample trees in the each level of thickness;
4) measurement of diameter of the sample tree accurate to 0.1 cm;
5) measurement of height of the sample tree accurate to 0.1 m;
6) cutting of the sample trees;
7) determination of volume of the sample tree by the formula:

$V_{aver} = g_{aver} \times h_{aver} \times f_{aver}$

where: g_{aver} – cross-sectional area of the average sample tree; h_{aver} – average height of the average sample tree, f_{aver} – form factor of the average sample tree;

8) determination of stock in the sampling unit according to the formula:

$M = V_{aver} \times N$

where: M – growing stock, V_{aver} – volume of the average sample tree, N – number of trees within the stand.

Values of the growing stock obtained on the sampling unit for investigated tree species was transferred on 1 ha according to the formula:

$M = M_{samp.un} \times F_{conv}$

by multiplying it on the conversion factor $F_{conv} = 1\ ha/S_{sampl.u}$.

According to the materials of forest management, there were found the area of black locust and Scots pine stands belonging to a particular age class, and the total growing stock of these species within these areas. The age class for black locust as a fast-growing species was 5 years, and 10 years for Scots pine. In the stands of the same age, determination of dependence of growing stock formation on the type of trophotope and hygrotope was determined by usage of the average growing stock by the formula:

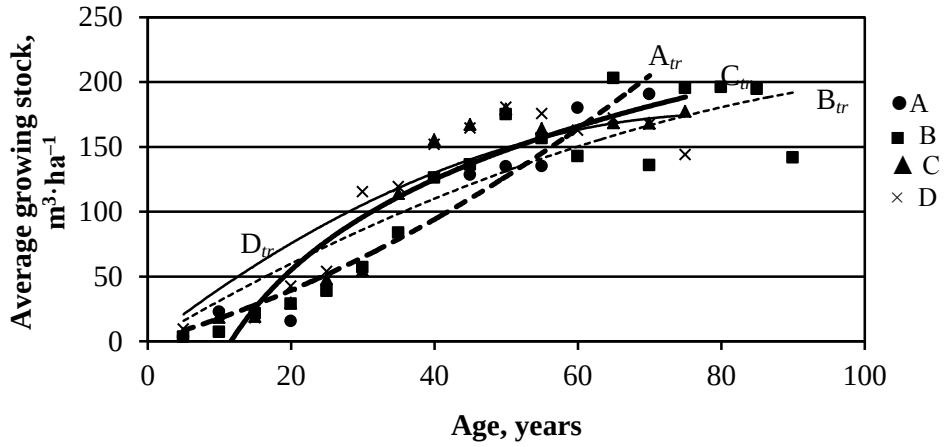
$M_{aver} = M_{total\ age\ cl}/S_{stand\ age\ cl}$

where $M_{total\ age\ cl}$ is the total growing stock in the same age class stands, $S_{stand\ age\ cl}$ is the area of the stand of given age class.

Results and discussion

Forest inventory data analysis allowed determining the current age structure of black locust stands; it was characterized by a significant predominance of overmature age group, which occupied 72.3 % (12786.0 ha) of the total black locust stands area, with a very small area of young stands (0.2 %, 375.2 ha). The age range was 5 to 95 years, average age was 43 years. According to variations of trophic graduation, the largest area of black locust stands (9596.5 ha, 54.3 %) was growing among suhrud, where it was located 53.1% of the total growing stock. The black locust stands occupied more than a third of total oak forest area (6732.2 ha, 38.1 %) with 40.8 % of total growing stock. The area of black locust stands is thinly represented under the poorest soil conditions within bir (139.8 ha, 0.8 %), where it is presented by the smallest part of growing stock (0.8 %).

The calculated values of black locust average growing stock demonstrated differences in age classes under different soil trophic conditions (Fig. 1).



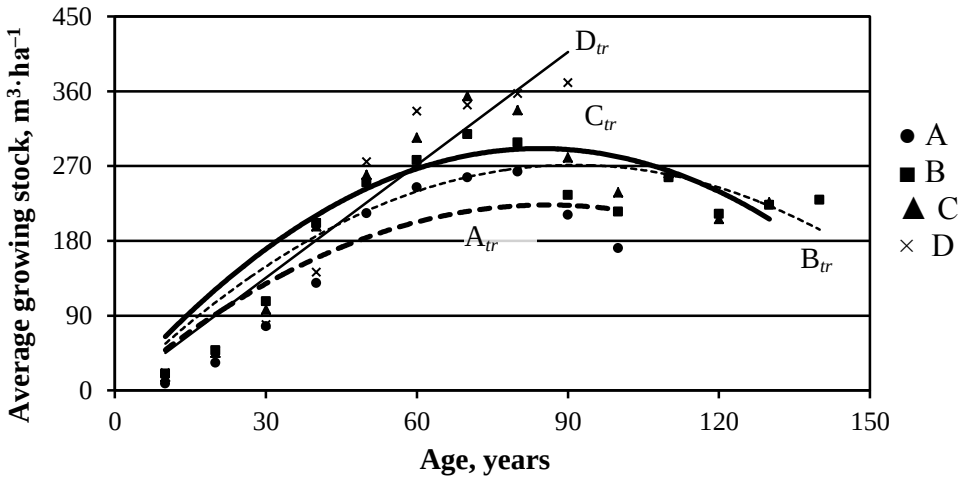
Note. Index *tr* – fitting trendline of growing stock in corresponding forest type.

Fig. 1 – Dependence of dynamics of the average growing stock on age of black locust stands in trophotopic sequence

Maximal values of average growing stock in trophotopes amounted: A – 180 m^3/ha^{-1} (12-th age class); B – 202 m^3/ha^{-1} (13-th age class); C – 178,9 m^3/ha^{-1} (10-th age class); D – 180 m^3/ha^{-1} (13-th age class). These data show the absence of significant differences in maximum average growing stock between the poorest soil conditions in the pure pine and oak forests, and the most fertile soils. It was noted decrease of the studied parameters, after attainment the age of 75 years for black locust (suhrud), 70 years (bir), and 65 years (subir and hrud).

Unlike black locust, ⅔ of the Scots pine stands area were concentrated in subir with the total growing stock 3017.66 $m^3 \cdot ha^{-1}$. Scots pine forms stands in hrud on the more small area (1.2 %). When soil fertility increases in pine stands from bir tosubir, respectively, it was experienced growth in the average growing stock value: it was 27.5 % more in trophotope D than A (Fig. 2).

In the pine stands, the highest values of average growing stock were registered in 8th age class within the bir andsubir, and in 7th class within the suhrud. Note that trend of gradual increase of stock amount in the hrud, before overmature 9th age class.



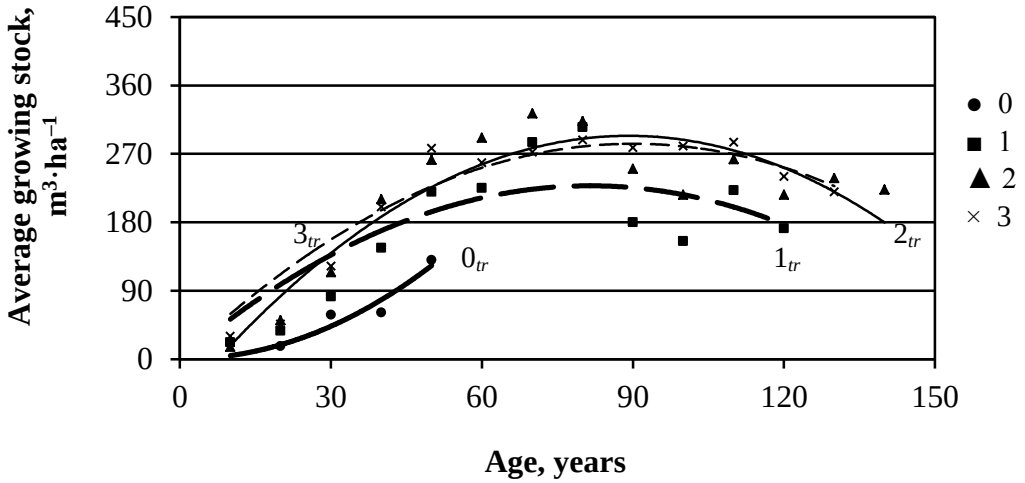
Note. Index *tr* – fitting trendline of growing stock in corresponding forest type.

Fig. 2 – Dependence of dynamics of the average growing stock on age of Scots pine in trophotopic sequence

With increasing the age, both for Scots pine and black locust, registered decrease of this index after attainment by pine the maximum growing stock in 8th and 13th age classes, respectively. It may be due to sanitary conditions deterioration within the stands, top-drying and twig blight.

Analyzing the area distribution of black locust stands depending on soil moisture conditions, it was found that the overwhelming majority was concentrated in the dry hygrotope conditions (13916.7 ha, 78.7 %). The area distribution in relation to moisture gradation was following: 1034.2 ha (5.8 %) of the stands area grows in the very dry hygrotope; 2594.1 ha grow in dry-mesic (14.7 %) and 95.5 ha (0.5 %) grow under wet condition. Compared to black locust, Scots pine forms the forest stands mainly in dry-mesic (12136.4 ha, 56.5 %) and dry (8817.1 ha, 41.1 %) hygrotopes, while the other moisture ranges had less than 3.0 % of the total pine forests area (Fig.3).

Determined average timber for different age of black locust stands in hygrotopes, it was found the studied index dependence on the moisture conditions: mean growing stock under wet conditions was 181.6 % compared to black locust stands occurred in the very dry hygrotope. Also, the similar trend is revealed for Scots pine, with a fixed predominance of average growing stock in mesic and wet habitat conditions in three times in comparison with the very dry conditions.

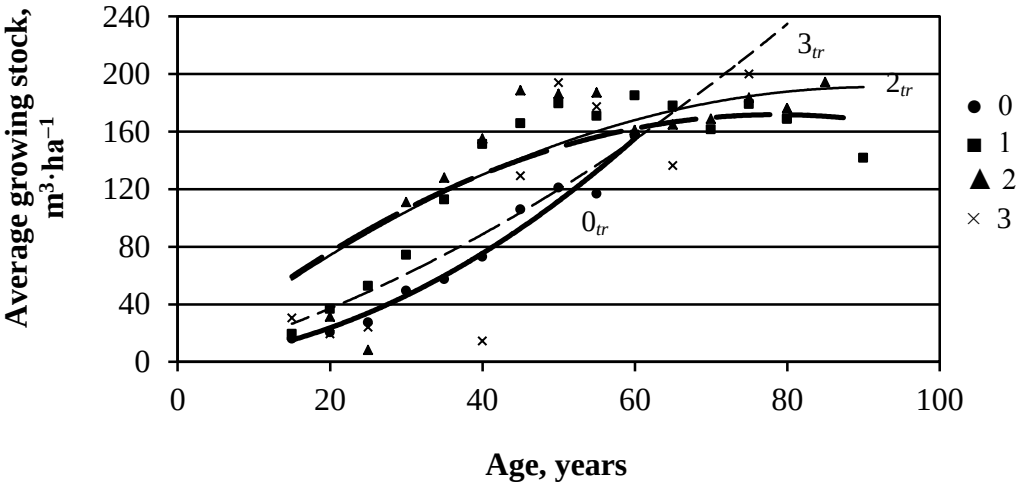


Note. Index *tr* – fitting trendline of growing stock in corresponding forest type.

Fig. 3 – Dependence of dynamics for the average growing stock on the age of Scots pine in hygrotopic sequence

It was observed increase the average value of growing stock in even-aged stands both Scots pine and black locust when changing of moisture conditions. However, it should be noted that this index decreased for pine in wet hygrotope, almost three-fold.

Black locust stands reach the maximum growing stock (193.9 m^3/ha^{-1}) in conditions of high moisture at the age of fifty years (10 age class). In different hygrogenic ranges, the following values of average growing stock were available for the same age: very dry – 121.0 m^3/ha^{-1}; dry – 179.4 m^3/ha^{-1}; mesic – 186.1 m^3/ha^{-1}, rather, it was observed the influence of moisture conditions on the studied indexes (Fig. 4).



Note. Index *tr* – fitting trendline of growing stock in corresponding forest type.

Fig. 4 – Dynamics of the mean growing stock from age of black locust in hygrotopic sequence

We determined that soil moisture conditions effect significantly on the yield of timber volume per hectare in Scots pine, as well as black locust. Scots pine reached maximum average growing stock (323 and 313 m^3/ha^{-1}) under mesic habitat conditions in the 7^{th} and 8^{th} age classes. The trend of this index increasing was not observed in different types of soil moisture, after 50 years in black locust stands, and 80 years in pine stands.

In Table 1 and 2, it was given the regression equations for average growing stock and its dependence on the age of stand in different gradations of tropho- and hygrotopes.

Table 1. Regression models of the average growing stock dependence on the age in different ranges of trophotopic sequence

Forest-forming species	Trophotope			
	A	B	C	D
Scots pine	$y = –0.0301x^2 + 5{,}19x$	$y = –0.0326x^2 + 5{,}95x$	$y = –0.0409x^2 + 6{,}90x$	$y = 4{,}526x$
	$R^2 = 0.79$	$R^2 = 0.81$	$R^2 = 0.80$	$R^2 = 0.91$
Black locust	$y = –0.0192x^2 + 1{,}58x$	$y = –0.0124x^2 + 3{,}24x$	$y = 100.9 \ln x – 247.3$	$y = –0.0264x^2 + 4{,}31x$
	$R^2 = 0.96$	$R^2 = 0.84$	$R^2 = 0.86$	$R^2 = 0.86$

Table 2. Regression models of the average growing stock dependence on the age in different ranges of hygrotopic sequence

Forest-forming species	Hygrotope			
	0	1	2	3
Scots pine	$y = 0.041x^{2.05}$	$y = –0.0343x^2 + 6{,}00x$	$y = –0.0351x^2 + 6{,}30x$	$y = –0.0349x^2 + 6{,}29x$
	$R^2 = 0.92$	$R^2 = 0.71$	$R^2 = 0.80$	$R^2 = 0.90$
Black locust	$y = –0.035x^2 + 0{,}485x$	$y = –0.0278x^2 + 4{,}37x$	$y = –0.0226x^2 + 4.16$	$y = 0.018x^2 + 1{,}50x$
	$R^2 = 0.97$	$R^2 = 0.80$	$R^2 = 0.74$	$R^2 = 0.71$

The values of determination coefficients explain 73-90 % of studied characteristics variability in Scots pine, and 91-94 % in black locust. Overall, the average growing stock in both studied species are characterized by significant variability due to influence of habitat factors, and it is necessary to continue surveys in this direction to such dependency study.

Conclusion

According to trophic graduation, the largest area of black locust stands was located within the suhrud, when half of the total growing stock is situated. Maximum value of the average growing stock of this species in trophotopes was 202 m^3/ha^{-1} (trophotope B, 13th age class). The differences of maximum values between average growing stock within bir and hrud were not registered.

The major part of the Scots pine stands area was located within the subir, when the total growing stock was 3017.66 m^3/ha^{-1}. When soil fertility increased, it was observed increase in the values of average growing stock in the Scots pine stands. In the pine stands, the highest values of average growing stock were registered in 8th age class within the bir and subir, and in 7th class within the suhrud. When age increasing in the Scots pine and black locust stands, it is observed maximum values of growing stock at the 8th and 13th age classes, and decrease of growing stock values at a later time.

Average growing stock of black locust under wet conditions was 181.6 % relative to growing stock produced under very dry conditions. A similar tendency was revealed in the Scots pine stands: average growing stock under wet-mesic and wet habitat conditions was three times higher than one under very dry conditions. As well as black locust, soil moisture conditions influenced significantly on volume of timber per hectare for Scots pine. This species reached the highest values of average growing stock (323 and 313 m^3/ha) under mesic conditions in 7th and 8th age ranges.

Under different moisture conditions, contrasting energy of growth of Scots pine and black locust caused significant differences in development and structure of their stands with age. Due to transition from optimum to the worst soil moisture conditions, it was observed decrease of shoot, fir needle and leaves formation, crown density, size of individual trees, and their resistance.

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